THE INFLUENCE OF SOME SPRAY MATERIALS ON THE INTERNAL STRUCTURE OF APPLE LEAVES

by

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INTRODUCTION

The purpose of this study was to discover (1) if certain spray residues affect the ratio of the internally exposed surface to the external surface of the leaves of certain varieties of apples, (2) if spray residues affect the internal structure or anatomy of apple leaves, and (3) some measurement that may be directly correlated with the ratio of the internally exposed surface to the external surface in order that the volume of necessary data and the time required would be reduced.

The apple varieties used in this study were selected so that comparisons could be made with the results of similar measurements in previous work by Pickett and Kenworthy (1939).

The general relation between leaf morphology and photosynthesis and the growth and fruiting of the apple was initiated as a project of the Kansas Agricultural Experiment Station by W. F. Pickett in 1932. Pickett (1932) studied the relationship between leaf area and size of fruit and in 1934, he reported on the correlation of internal structure of apple leaves to their photosynthetic activity. In 1937, Pickett studied extensively the relation of internal structure and photosynthetic behavior of apple leaves. Turrell (1936) suggested a formula by which one might mathematically express the

ratio of the internal exposed surface to the exterior surface of foliage leaves. Using this formula, Pickett and Kenworthy (1939) investigated the relationships between structure, chlorophyll content, and photosynthesis in apple leaves.

The photosynthetic activity of apple leaves and the effect of certain spray residues on the rate of photosynthesis has been the subject of much research in recent years. The rate of photosynthesis of apple leaves was studied by Heinicke and Hoffman (1933) who used a special carbon dioxide assimilation chamber in this work, the principle of which had been used with more or less modification by many workers since Kreusler first suggested it in 1885. Heinicke (1937) studied the effect of a lime sulphur 1-40 spray on the rate of photosynthesis of an entire ten-year-old Baldwin apple tree. In 1938, he investigated the influence of sulphur dust on the rate of photosynthesis of an entire apple tree. Hoffman (1932) reported on the effect of certain spray materials on the carbon dioxide assimilation by McIntosh apple leaves, and in 1933, he reported on the carbon dioxide assimilation by apple leaves as affected by lime sulphur sprays. He studied the effect of lime sulphur spray on the respiration rate of apple leaves in 1935. Christopher (1935) investigated the effect of flotation sulphur spray on the carbon dioxide assimilation of apple leaves. Brody and Childers (1938) reported on the effect of dilute liquid lime sulphur sprays on the photosynthesis of apple leaves. Agnew and Childers (1939) studied

the effect of two mild sulphur sprays on the photosynthetic activity of apple leaves, and in 1939, Southwick and Childers reported on the influence of Bordeaux mixture on the rate of photosynthesis and transpiration of apple leaves.

REVIEW OF LITERATURE

General Anatomy of Dorsiventral Leaves

Eames and MacDaniels (1925) etated that the photosynthetic tiesue, which consists typically of thin-walled parenchyma, between the upper and lower epidermis of dorsiventral leaves, is known as mesophyll. This tissue constitutes the major portion of the substance of the leaf. Palisade mesophyll is the name given to those compact, elongate, more or less cylindrical cells which are near the upper epidermis and lie perpendicular to the leaf surface. Palisade mesophyll may develop on both sides of a leaf that stands more or less vertically or assumee a drooping position. Those cells, loosely arranged and irregular in shape, near the lower epidermis are known as the spongy mesophyll. The cells of this spongy mesophyll with their radiating arms connect with each other to form a loosely arranged network, which connects with the emall near-by branches of the veins and also with the lower epidermis through which gas passes by way of stomata.

Haberlandt (1928) stated that the palisade mesophyll is

recognized as the most important of the specialized photosynthetic tissue of the leaf. The spongy mesophyll, connecting the photosynthetic tissue with the different channels, constitutes the physiological link. Photosynthesis is a secondary function of the spongy mesophyll, although these cells do contain a few chloroplasts. The products of photosynthesis are diffused through the first layer of palisade mesophyll to the layer beneath it, and so on to the spongy mesophyll which carries it to the minute branches of veins. Thus each palisade cell functions independently of the adjacent cells of the same layer of mesophyll.

According to Delisle (1938) the shape and size of a leaf within a species are due both to cell number and cell enlargement. The shape of the leaf is due in part to factors limiting the number of cells and the direction of cell enlargement, rather than to the differences in cell shape.

Differentiation in Dorsiventral Leaves

According to Haberlandt (1928) the differentiation of palisade cells is always initiated by the appearance of active anticlinal division in approximately isodiametric mother-cells, and the palisade cells never arise from the mere elongation of isodiametric meristem elements. These partitions appear at different stages of development in different plants. The palisade tissue appears contemporaneously with the small veins

in Ficus elastica Roxbg. The palisade tissue appears after the segregation of the principal veins and before the segregation of the smaller veins or vascular bundles in Caragana frutescens DC.

Working with Vitis vulpina L. and Catalpa bignonioides Walt., Mounts (1932) concluded that the intercellular space was schizogenous in origin. He said that the cell layers begin to differentiate into epidermis, palisade and spongy mesophyll when the blade is from five to eight millimeters long. An important factor in the development of the intercellular space is the more rapid expansion of the epidermal layers, tending to separate the cells of both the palisade and spongy mesophyll.

Avery (1933) worked with tobacco plants and found that cell divisions cease first in the epidermis, followed by the spongy mesophyll, and then in the palisade. He reported that the uniform spongy condition resulted from the strain of the expanding epidermis on cells of the middle and lower mesophyll, together with the fact that these mesophyll cells cease dividing and their rate of enlargement is insufficient to meet the strain, causing them to pull apart. Layers of the palisade and spongy mesophyll were multiplied in a plane parallel to the surface of the leaf. When the leaf was 1/80 to 1/75 of its final size, the cells of the palisade mesophyll began to acquire their characteristic shape. Intercellular space did not develop markedly until the leaf was from 1/4 to 1/3 of its

final size. The differential distribution of growth in its various portions determines in part the final shape attained by the leaf as a whole.

Factors Affecting the Anatomy of Leaves

Light. Eames and MacDaniels (1925) stated that light intensity has a great influence on the number of palisade layers and the density of the cell structure. Bergen (1904) reported that sun leaves of evergreen angiosperms were thicker than the shade leaves; the cells next to the epidermis were longer in the sun leaves. A palisade layer occasionally developed next to the lower epidermis of sun leaves, as sun leaves were often vertical while shade leaves were parallel to the ground. The intercellular space was less in the upper portions of the mesophyll of sun leaves. The bundles were much more highly developed in sun leaves. Sun leaves were usually narrower than shade leaves in proportion to their length. The xerophytic leaf structure is not always incompatible with abundant transpiration, but sometimes exists only for use in emergencies to protect the plant from injurious loss of water.

Turrell (1933) reported that succulent leaves may have a relatively small internal surface, (R = 7.86), and that xeromorphic leaves of shade species may have a limited internal surface, (R = 8.18 to 9.88), mesomorphic sun leaves, though thin, may have a relatively large internal surface,

(R = 11.6 to 16.3), while xeromorphic leaves of sun species may have an extensive internal surface, (R = 22.2 to 31.3). "R" represented the ratio of the internally exposed surface to the external surface of leaves.

According to Turrell (1936), again using "R" to represent the ratio of the internally exposed surface to the external surface, shade leaves have a relatively small internal surface, (R = 6.8 to 9.9), intermediate internal surface for leaves of mesomorphic type, (R = 11.6 to 19.2), and high for xeromorphic sun leaves, (R = 17.2 to 31.3).

Hesselman (1904) stated that leaves of forest trees grown in the stronger light had more palisade cells than those in the poorer light. When the light was equal, shade leaves produced more starch than sun leaves of the same species.

Pfeiffer (1928) found that the leaves of four-o'clocks, sunflowers, and soybeans developed two rows of palisade cells outdoors, while only one row developed in the shade. The leaves decreased in thickness in the order of the following degrees of illumination: full sunlight, full spectrum, visible spectrum, minus violet, blue shade, and red. According to Penfound (1931, 1932), the leaves of sunflower, water pepper, and castor bean had a better development of mesophyll and were thicker when grown in full sunlight than when grown in shade. Clements and Long (1935), working with Helianthus, reported that the palisade tissue consistently composed more than 50 per cent of the leaf thickness, and that the greater

the per cent of illumination, the greater the thickness of the leaf. That leaves of Cornus florida L. were somewhat thicker on the south side than on the north side of the tree was reported by Shank (1938) who also found that leaves were thicker and somewhat smaller in the open than in the woods. Further effects of light were found by McDougall and Penfound (1928) who reported that leaves from dense shade were thinner, had more surface, with less palisade, and a higher per cent of intercellular space and spongy mesophyll than leaves of the same plant in maximum sunlight.

Clements (1904) reported that an increase in thickness of leaf and a somewhat looser arrangement of mesophyll cells resulted from decreased light. According to Lundegardh (1931), certain trees, such as birch and ash, are unable to produce typical shade leaves. He found that leaves growing in shady places were thin and poorly differentiated, while bright light produced thick, well differentiated leaves. In comparing the same species of plants at various elevations in the Alps and Pyrenees, Bonnier (1894) found that the Alpine leaves had a better developed palisade tissue due to larger cells or an increase in the number of rows. Kenworthy (1939) found that the ratios of the internally exposed surface to the external surface were greater for field leaves than for those grown in the greenhouse.

It would seem from the above findings that light has an extremely pronounced effect on the anatomy and morphology of

foliage leaves. Some leaves are inheritantly adapted to sun or shade and are thin or poorly differentiated when placed under the opposing condition, but in the majority of cases leaves are thicker when in full sunlight than in shade, and sometimes an extra layer of palisade mesophyll is actually formed.

Position of Leaf. Eames and MacDaniels (1925) stated that there is often considerable variation in the mesophyll structure of leaves from different parts of the same plant.

Concerning variations in leaf thickness, Cowart (1935) found that leaf thickness decreases from the base toward the median portion of the shoot and then increases from that point to the apex of the shoot. There was an increase of palisade tissue from the base of apple shoots to the tip, also a parallel increase in per cent of palisade mesophyll and a decrease in intercellular space in the mesophyll. He found a negative relationship between these characters and vigor of shoots.

A statement of "Zalensky's law" was given by Moissejewa (1938) concerning the position of the leaf on the tree in which he said, "Zalensky, studying monocotyledonous and dicotyledonous plants on a very extensive and variegated material, showed that the higher up on the tree the leaf grows (or the nearer the end of the branch), the greater the xeromorphic properties it acquired; that is, the epidermis and mesophyll cells are smaller, the conducting strand is thicker, the stomata are greater in number and smaller, and the

palisade tissue is more clearly defined." A similar law was found for Gymnospermae.

Working with Tilia europea L., Ewart (1906) removed all lateral buds from the shoots, leaving only the terminal buds. The terminal buds produced leaves that were much larger than those which grew on normal plants. Their cells were of the same size as those of ordinary leaves, however, indicating that leaf enlargement was due to cell division and that this plant is characterized by a constant normal cell size. Leaves nearer the ground were larger than those higher in the air or on the stem, according to Yapp (1912), who worked with plants of a marsh. Leaves at different nodes of sunflower and water pepper plants differed with respect to thickness of leaf, and depth of palisade and spongy mesophyll (Penfound, 1931).

It may be concluded from the foregoing literature that leaves differ in mesophyll structure from different parts of the plant, but the differences are variable and cannot be foretold, as some plants have larger leaves at the apex, and others are larger at the base.

Soil Moisture. Increased water caused a decrease in thickness and a looser arrangement of the mesophyll cells, especially of the sponge cells, according to Clements (1904). A decrease in water caused an opposite effect.

Clements and Long (1935), working with Helianthus, showed that the palisade tissue consistently composed more than 50 per cent of the leaf thickness, and that the greater the per cent

holard, the greater the thickness of leaf.

Penfound (1931, 1932) found that the leaves of sunflower, water pepper plants, and castor bean were thicker when the plants were grown in soil of a high water content than when grown in soil of a low water content. The number of rows of palisade and sponge cells was constant under all soil moisture conditions, but the palisade and spongy mesophyll were deeper if grown in soil of high moisture.

Inheritance or Varietal Differences. Tenopyr (1918) stated that cell size depends upon the time or stage of development; the later appearing leaves having smaller cells. The average cell size for any one tissue of a species or variety is a fairly constant and hereditary character, though the cells of plants vary considerably in size in the same tissue.

According to Halma (1929), the Eureka and Lisbon lemons had three rows of palisade cells, while the other species possessed only two rows of palisade cells. The percentage of palisade tissue varied with age of leaf and illumination, but was practically constant for a species, ranging from 20.9 to 31.9 per cent. He found that the rate at which the cuttings take root follows very closely the order of the degree of palisade development. When root cuttings possessing a similar leaf area were planted in the nursery, the rate of subsequent growth followed the same general order as given for the palisade development. According to Halma, Languer found that the

palisade tissue is two to three times as thick in a sun leaf as in a shade leaf of Acer pseudoplatanus, and Alexandrov found that in grapes, the variety having the greatest number of palisade cells is the most productive and the one with the least number, the least productive.

Pickett (1933) reported the rating of Livland, Wealthy, York, Winesap, Gano, Jonathan, and Delicious varieties of apples in ascending order of compactness of mesophyll, and the perimeter of the intercellular space in the spongy mesophyll. Pickett (1934) found that orchard grown Livland leaves had more extensive intercellular space than orchard grown Delicious leaves and that field leaves of Livland and Delicious trees had greater intercellular space than greenhouse leaves. Livland, Delicious, Jonathan, and York were rated in ascending order by Pickett (1937) on the basis of the extent of intercellular space as judged from tracing projected images of cross sections.

Using measurements from camera lucida drawings and Turrell's formula, Kenworthy (1939) reported the rating of Livland, Jonathan, York, Wealthy, and Winesap varieties of apples in ascending order of extent of the ratios of the internally exposed surface to the external surface for field grown leaves. For greenhouse grown leaves, he rated them York, Jonathan, and Wealthy in ascending order.

Miscellaneous Factors. According to Hill (1934) the cells of the palisade mesophyll of leaves of potato plants

affected with giant hill were smaller than those of healthy plants, with a smaller ratio of width to length, and smaller volume. The leaves of the diseased plants were thinner than those of the healthy plants.

Lutman (1934) reported that a study of rape plants showed the length of palisade cells shortened when any variation was made from a complete solution, and buckwheat showed a greater variation than rape. The leaves of the potato grown with excess nitrogen were crowded with small, short cells with relatively small air spaces; while with an absence of nitrogen the leaves were spongy, and had large intercellular spaces and long slender cells. In general, the size of plants was found to be correlated with that of the cell and cellular organs.

Noguchi (1935) and Wolcott (1936) changed the anatomical structure of leaves with the X-ray. Noguchi found that the size and shape of palisade tissue may be disrupted to the extent that it cannot be distinguished from the spongy parenchyma. The disturbance increased with the time of irradiation.

Pickett (1937) found that leaves of Livland, Jonathan, and York varieties of apples had a greater extent of intercellular space in a warm house than in a cool house, while the reverse was found to be true with the Gano.

Factors Affecting the Photosynthetic Activity of Apple Leaves

It has long been known that a great many factors affect

the photosynthetic activity of foliage leaves. Miller (1938) stated that the factors affecting photosynthesis include the carbon dioxide supply, light, temperature, water supply, chlorophyll content, and the protoplasmic factors.

Christopher (1934) stated that during the late fall many of the leaves on an apple tree of bearing age receive very low light intensities. He suggested that light may be an important limiting factor to photosynthesis in orchards, especially where pruning is neglected. According to Christopher (1933) the leaves on the east side assimilate about twice as much as those on the west side during the early morning. There is less difference during the midday and a tendency for the leaves on the west side to do a little better during the late afternoon. He said that the leaves exposed to the sun during the morning are in a position from the standpoint of water supply, carbon dioxide supply, and temperature, to take fullest advantage of the available light, as there is not apt to be incipient wilting, and the stomata are open for a longer period of time. Christopher (1938) stated that a normal leaf on thin wood may assimilate carbon dioxide as a rate equal to or greater than a similar leaf on thick wood, and suggested that light is a determining factor controlling the carbon dioxide assimilation of these thin wood leaves in some instances.

The internal conditions which govern the supply of water and nutrients and the translocation and utilization of

assimilated materials seem to have a profound influence on the efficiency of the foliage of the apple as well as that of other plants, according to Heinicke and Hoffman (1933). They suggested that this may throw some light on red color and alternate bearing. Color, sprays, soil, water supply, ringing the stem, weather conditions, susceptibility to winter injury, blight, and aphids all have effect on photosynthesis.

Heinicke and Childers (1936) stated that the average rate per hour of apparent respiration of an entire apple tree during the night period amounts to considerably less than 10 per cent of the average hourly rate of apparent photosynthesis during the day. The rate of apparent photosynthesis during the day, however, may be depressed 20 to 30 per cent by respiration. Waugh (1939) found that under fairly uniform external conditions, the rate of assimilation of apple leaves is irregular. He felt that internal factors play a significant part in the assimilation of the apple leaf.

Pickett (1933) suggested that the superficial area of the intercellular boundaries may be a factor at least partly governing the rate of absorption of carbon dioxide and thus influencing the rate of photosynthesis, as the carbon dioxide entering through the stomata diffuses through the intercellular spaces to the moist surfaces of the mesophyll where it is absorbed. According to Pickett (1934) orchard grown Livland leaves have more extensive intercellular spaces than orchard grown Delicious leaves and apparently these

differences are reflected in the photosynthetic behavior of the two varieties. He suggested that the more open mesophyll of the orchard grown leaves may be one of the contributing factors in enabling them to be more active in carbon assimilation than the greenhouse grown leaves. The extent of the internally exposed surface of apple leaves is more important than the chlorophyll content as a factor partially governing photosynthetic activity, according to Pickett and Kenworthy (1939).

Effect of Spray Materials on the Photosynthetic Activity of Apple Leaves

Hoffman (1932), working with McIntosh apple leaves and using the carbon dioxide assimilation chamber, reported that leaves sprayed with lime-sulphur 1-40 show a reduction of 37.1 per cent of their former photosynthetic activity, and that light green leaves were reduced in efficiency much more than the dark green leaves. Bordeaux showed very little to no injury, while a commercial brand of summer oil reduced carbon dioxide intake appreciably. According to Hoffman (1933), again working with lime-sulphur sprays, one leaf was reduced 41 per cent in its efficiency at 29° C. while the check deviated 5 per cent. Leaves sprayed at 1:40 p. m. showed a greater reduction than those sprayed at 6:45 p. m. He reported some marginal burning on leaves sprayed at 1:40 p. m., but not when sprayed at 6:45 p. m. New Jersey dry-mix was

not as harmful as 1-40 lime-sulphur. Hoffman (1935) stated that lime-sulphur injury to apple leaves is largely due to a decrease in the rate of photosynthesis rather than an increase in the rate of respiration. The decrease in the rate of photosynthesis will at least much more than offset any increase that may take place in the rate of respiration. He stated that there seems to be a tendency for the sprayed leaves to show a slightly greater rate of apparent respiration than the untreated leaves.

Christopher (1935) reported that flotation sulphur may cause a reduction in the carbon dioxide assimilation of apple leaves, but the reduction is not nearly as serious as that reported by Hoffman when lime-sulphur is used. He stated that flotation sulphur, from the point of view of its effect on carbon dioxide assimilation, may therefore be considered safer than lime-sulphur as an apple scab spray.

Heinicke (1937), using a lime-sulphur 1-40 spray on an entire ten-year-old Baldwin apple tree, found that during the 5 days after the first spraying on July 6-7, the foliage of the treated tree was only about one-half as active as during the preceding 6 days, while at the same time the check tree showed a slight increase in average daily rate. He stated that the reduction in rate of photosynthesis caused by the spray during this 5-day period alone represented a loss of dry matter equivalent to that found in about 1/2 bushel of mature apples. Heinicke (1938) stated that finely divided sulphur

dust, as compared to lime-sulphur solution, has relatively little influence on the rate of photosynthesis of the leaves of an entire tree.

Brody and Childers (1938) stated that the dilute liquid lime-sulphur sprays may cause marked reductions in the apparent rate of photosynthesis of Stayman apple leaves for 3 to 5 days after spray treatment, even though no visible burning occurs. They also observed that when the maximum temperature reaches 90° to 100° F. a significant reduction in assimilation usually occurs regardless of the spray concentration in contact with the leaf tissues.

Agnew and Childers (1939) reported that sprays which contain sulphurs in suspension have less effect on photosynthesis of apple leaves than sprays which contain sulphurs in solution.

Bordeaux mixture reduces the rate of photosynthesis and transpiration of apple leaves more at higher temperatures, according to Southwick and Childers (1939). Murphy (1939) reported that from the standpoint of photosynthetic efficiency, Cupro K is superior to Coposil, 6-8-100 Bordeaux, and lime-sulphur as a spray for sour cherries.

The above literature would indicate that spray materials definitely cause a reduction in photosynthetic activity of foliage leaves, and the reduction by the more caustic sprays is greater than that caused by the mild forms. Temperature is also a factor, more damage resulting at higher temperature.

Intercellular Space and Internally Exposed Surface of Leaves

That there is intercellular space and a large internally exposed cell-wall surface in contact with this internal atmosphere in foliage leaves is a fact recognized by all the workers in the field today.

Eames and MacDaniels (1925) stated that the normal structure of the mesophyll is such that a large cell-wall surface is exposed to the "internal atmosphere" of the leaf. The proportion of wall surface of the mesophyll cells exposed to the intercellular spaces varies greatly in different plants, depending on the density of the mesophyll, which varies with the habitat of the plant.

Turrell (1934) prepared formulas for the measurement of the internally exposed surface of mesomorphic, strongly zeromorphic, and succulent leaves. The ratio (R) of the internally exposed surface to the external surface could be determined from these formulas. This formula for the measurement of the internally exposed surface and calculation of R for a mesomorphic leaf is as follows:

$$R = \frac{\Sigma(ab\ldots a_n b_n) + l(cd + \frac{2ef}{g}) + \frac{hi}{j}}{2K}$$

In the foregoing formula, the part "$(ab\ldots a_n b_n)$" represents the surface exposed in the palisade region;

"$l(cd + \frac{2ef}{g})$" represents the exposed surface in the spongy mesophyll; "$\frac{hi}{J}$," represents the exposed surface of the lower epidermis; and "2K" represents the external surface. All measurements represented by letters were made from camera lucida drawings with a planimeter and chartometer.

Formulas for measurement of the internally exposed surface of leaves of the same types of structure in another form were given by Turrell (1936). His new formula for the measurement of the internally exposed surface and calculation of R for a mesomorphic leaf follows:

$$R = \frac{\Sigma lp + L(hc + 2A\frac{l_e}{l_t}) + (K^2 - A)\frac{l_i}{K}}{2K^2}$$

For explanation of this formula see page 27. The exposed surface of the upper epidermis which, at most, represents 0.2 per cent of the internally exposed surface, according to Turrell, is not taken into consideration with this formula. The formulas were based on calculations from a theoretical leaf, and the measurements were taken from camera lucida drawings. He checked the accuracy of the instruments used.

Turrell stated that the two formulas are precisely the same.[1] The empirical use of letters in the preliminary paper

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[1]Correspondence with Mr. A. L. Kenworthy, November 30, 1937.

was changed in the later publication for letters more suggestive of the measurements which they represented. The part of the formula lp means $lp + l_1p_1 + l_2p_2 \ldots$ He stated also that "The use of an oil immersion objective is essential. Projection with an 'Edinger' projector was not as satisfactory as the camera lucida".

Turrell (1936) determined the internally exposed surface of several genera and species, and the ratio of the internally exposed surface to the external surface was computed. He discovered that a leaf has the largest percentage of internally exposed surface in the palisade region. Ratios representing the range of his work were from a low of 6.8 for shade leaves to a high of 31.3 for xeromorphic sun leaves.

Kenworthy (1939), using Turrell's formula for the calculations, and a chartometer and planimeter from camera lucida drawings for the measurements, made a study of the internally exposed surface of foliage leaves of five varieties of apples. He stated that the formula used in this study can be used to determine the ratio of the internally exposed surface to the external surface of apple leaves. He found that the palisade mesophyll contained 85 per cent or more of the total internally exposed surface of apple leaves. There was a highly significant correlation between the total depth of palisade mesophyll and the ratio of the internally exposed surface to the external surface. There was a general decrease in the per cent of the total internally exposed surface as the region

considered became more distant from the upper epidermis.

MATERIALS AND METHODS

Greenhouse and Field Leaves

On January 29, 1940, 22 two-year-old trees of Wealthy and 21 two-year-old trees of York varieties of apples were planted in 12-inch clay pots, and placed into a ground bed in a greenhouse.

Spray Schedule for Greenhouse Leaves. Soon after the buds started to grow, eleven trees of Wealthy and ten trees of York were sprayed with two and one-half gallons of liquid lime-sulphur and four pounds of lead arsenate per hundred gallons of water. This spray was repeated at weekly intervals. Relative humidity readings were taken at each application and the temperature was regulated so as to leave as heavy a residue as possible with no visible injury. Research workers have shown that higher temperatures are conducive to more injury, and vice versa.

On March 28, 1940, two-year-old trees of Wealthy, Jonathan, and York varieties of apples were planted in the Horticultural gardens northeast of Dickens Hall. Ten trees each of Jonathan and York, and six of Wealthy were planted.

Spray Schedule for Field Leaves. When the buds on the

field trees had started to grow, one-half of the trees of each variety were sprayed at weekly intervals and the rest were left unsprayed. These sprays consisted of six applications of four pounds of lead arsenate and two and one-half gallons of liquid lime-sulphur per hundred gallons of water, three applications of four pounds of lead arsenate alone per hundred gallons of spray, and five applications of four pounds of lead arsenate and two gallons of oil per hundred gallons of spray.

Collecting and Imbedding of Leaves. On April 29, 1940, five apparently uninjured leaves were collected from each of the greenhouse trees, the leaves selected being from near the middle of the new shoots and of average size for the tree.

On July 1, 1940, after the last spray in which lead arsenate alone was used, and on August 5, 1940, at the end of the season, five leaves were collected from each of the field trees in the same manner as for the greenhouse trees.

Portions of the leaf used for study were located near the midrib and midway between the basal and apical regions. The marginal and midrib portions of each leaf were discarded. Only one piece, about one by three centimeters, was taken from a single leaf. These leaf pieces were placed in a one per cent chromo-acetic acid killing and fixing solution. After leaving them in the killing and fixing solution for 24 hours, they were washed then dehydrated with N-butyl alcohol (Zirkle, 1930) after which the leaf pieces were imbedded in paraffin (Brough, 1939).

Preparation of Slides. Five sets of slides were made for each tree. A set of slides consisted of one slide with cross section and one slide with tangential section, each made from the same leaf piece, which was oblong to facilitate the making of these two slides. All sections were eight microns thick, fixed on the slides with egg albumin after having been floated on warm water containing potassium dichromate. They were then stained with one-half to one per cent safranine O in 50 per cent alcohol, and mounted with balsam. Three to five sections were placed on each slide.

Drawings. Two pages of drawings were made from each set of slides. Four hundred thirty pages of drawings were made for the total. Each page of drawings included the following: one drawing of a field 50 microns square of each of the first, second, and third layers of palisade cells from a tangential section; one drawing of a field of the spongy mesophyll 50 microns square, from a tangential slide; and one drawing of a field 50 microns wide, across the spongy mesophyll, in cross section (Fig. 2). These drawings were arranged across the page and from the top to the bottom in the order mentioned. The drawings were made by using a camera lucida, the microscope was fitted with a 1.9 mm. oil immersion objective and a 10x eyepiece with the mirror arm at 120 mm. This produced a magnification of approximately 1760.

At first it was rather difficult to determine which layer of palisade cells was in the field when making the tangential

drawings. Cross sections of each variety showed that the epidermis dipped where veins were present, and that all leaves of the Wealthy contained three layers of palisade cells, but in a large percentage of the York, the third layer of palisade cells were extremely few in number or missing altogether (Figs. 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12). Above small veins the epidermis was found in a number of layers, sometimes as many as five or six layers of cells above the parenchyma cells surrounding the bundle, while above large veins the epidermis consisted of a single layer above the parenchyma cells surrounding the bundle. This characteristic of the epidermis was used in determining the layer number of palisade cells, in tangential sections, as outlined below.

Palisade cells were considered to be of the first layer when found in a microscopic field adjacent to cells which were definitely of the upper epidermis but not near a vein. The first layer of palisade cells was also considered to be those found in fields which showed vein tracings of epidermal like cells, but including no tracheids (Figs. 13, 14, 15, 16, 17, 18, 19, and 20).

Cross sections of the leaves of both varieties all contained druses or inclusions which were in the second layer of palisade cells only. Such druses or inclusions were significant in locating fields of the second layer of palisade cells. Also microscopic fields which contained veins with tracheids that disappeared toward the upper palisade cells were

considered to be of the second layer (Figs. 21, 22, 23, 24, 25, 26, 27, and 28).

When cross sectional measurements showed no third layer of palisade cells, or too few to be counted, no tangential drawings were made. When present, the third layer was determined in a similar manner as the second layer. If the cells of a microscopic field in the palisade region contained veins with tracheids which disappeared when the field was moved toward the spongy mesophyll, they were considered to be of the third layer. The third layer of palisade was always less compact than the first and second layers; this facilitated differentiation between the second and third layers of palisade cells (Figs. 29, 30, 31, 32, 33, 34, 35, and 36).

Microscopic fields that were free of veins were used for drawings of the spongy mesophyll (Figs. 37, 38, 39, 40, 41, 42, 43, and 44). Regions that showed the lower epidermis intact were used for the cross sectional drawings. Palisade cells were drawn and labeled as such when in contact with cells of the spongy mesophyll, but were not measured. True tangential sections were difficult to prepare due to the universal though slight warping of the leaf pieces in the process of imbedding, but this warping made possible the drawing of all tangential layers from a single slide. In order to eliminate using the tips of palisade cells, areas which were uniform in cell size were used in making the drawings.

Measurements. Measurements of the drawings were made with a chartometer and a planimeter. The following formula used by Turrell (1936) for computing the ratio (R) of the internally exposed surface to the external surface of mesomorphic leaves, required these measurements:

$$R = \frac{(lp) + (l_1p_1) + (l_2p_2) + L(hc + 2A\frac{l_e}{l_t}) + (K^2 - A)\frac{l_i}{K}}{2K^2}$$

The following symbols, stated briefly, represent the foregoing measurements:

- p - Exposed perimeter of upper palisade cells in tangential section;
- p_1 - Exposed perimeter of second layer of palisade cells in tangential section;
- p_2 - Exposed perimeter of third layer of palisade cells in tangential section;
- l - Average length of 10 cells, in cross section, of the upper palisade cells; measured directly with eyepiece micrometer;
- l_1 - Average length of 10 cells, in cross section, of second layer of palisade cells. Measured directly with eyepiece micrometer;
- l_2 - Average length of 10 cells, in cross section, of third layer of palisade cells. Measured directly with eyepiece micrometer;
- L - Average number of tiers of cells in the spongy mesophyll, in cross section;
- A - Average area of cells of spongy mesophyll in tangential section;
- c - Avorage length of the exposed cell wall in tangential section of the spongy mesophyll;
- h - Average length of vertically exposed cell walls in spongy mesophyll of cross section;
- l_e - Total length of exposed cell walls making an angle greater than 45 degrees with the vertical in cross section of the spongy mesophyll;
- l_t - Total length of exposed and non-exposed cell walls making an angle greater than 45 degrees with the vertical in cross section of spongy mesophyll;
- l_i - Average length of inner wall of lower epidermis in cross section;
- K - Constant, length of one side of sample area.

All measurements except l, l_1, l_2, and A were recorded in centimeters. The measurements l, l_1, and l_2 were recorded in microns, and measurement A was recorded in square inches. All measurements were transposed to microns or square microns before computing R. The measurements l_e, l_t, l_i, and K were used in ratios of $\frac{l_e}{l_t}$ and $\frac{l_i}{K}$ and these ratios were computed from the centimeter measurements.

The following tissue measurements may be computed from the formula: the internally exposed surface of the palisade, $(lp) + (l_1p_1) + (l_2p_2)$; the internally exposed surface of the spongy mesophyll, $L(hc + 2A\frac{l_e}{l_t}) + (K^2 - A)\frac{l_i}{K}$; the horizontally exposed surface of the spongy mesophyll, $L(hc)$; the vertically exposed surface of the spongy mesophyll, $L(2A\frac{l_e}{l_t})$; and the exposed surface of the lower epidermis, $(K^2 - A)\frac{l_i}{K}$.

The foregoing measurements were substituted in the formula and the ratio (R) of the internally exposed surface to the external surface was computed for each individual page of drawings. In Table 1 are presented measurements taken from the drawings in Figure 1.

The number of cells in the cross section drawings of the spongy mesophyll was needed to compute the average vertically exposed cell wall (h), and the number of cells in the tangential drawings of the spongy mesophyll was needed for the

computation of the average exposed surface (c). These values were given no symbol in the formula as they were used in determining the value of the above measurements before they were used in the formula.

The outside diameter of the upper layer of palisade cells was measured and given symbol D. These measurements were taken from the drawings in microns. The average number of cells of the upper layer of palisade in each drawing was also computed and given the symbol N.

Table 1. Measurements and calculations of "R" for drawings in Figure 2.

Symbol	Centimeters	Microns	Symbol	
p	98.0	589.96	l	62.52 microns
p_1	97.0	583.94	l_1	42.48 microns
p_2	78.0	469.56	l_2	33.36 microns
c	6.8	40.94	A	196.35 microns
h	5.5	33.11	L	5.00 av. no. cells

$$\frac{l_e}{l_t} = \frac{33.7}{37.5} = 0.90 \qquad \frac{l_1}{K} = \frac{8.9}{8.3} = 1.07$$

Calculation of R

Area in palisade $= \Sigma lp = l \times p + l_1 \times p_1 + l_2 \times p_2$

$= 62.52 \times 589.96 + 42.48 \times 583.94 + 33.36 \times 469.56$

$= 77,354.59$ sq. microns

Area in sponge $= L(hc + 2A\frac{l_e}{l_t}) + (K^2 - A)\frac{l_1}{K}$

$= 5(33.11 \times 40.94 + 392.7 \times 0.90) + (2500 - 196.35)\ 1.07$

$= 11,009.66$ sq. microns

$$R = \frac{\Sigma lp + L(hc + 2A\frac{l_e}{l_t}) + (K^2 - A)\frac{l_1}{K}}{K^2}$$

$$= \frac{77,354.59 + 11,009.66}{5000}$$

$= 17.67$

PRESENTATION OF DATA

Greenhouse Leaves

Measurements of leaves of Wealthy and York trees planted in the greenhouse were made as described in the materials and methods section. The ratio of the internally exposed surface to the exterior surface, "R"; the total depth of the palisade layers, "P"; the average number of upper palisade cells per 2500 square microns of leaf area, "N"; and the average diameter of the first layer of palisade cells, "D" are recorded in Table 2.

Table 2. Average R, P, N, and D for greenhouse grown leaves, 1940.

Variety	Treatment	R	P in microns	N	D in microns
Wealthy	check	13.40	104.49	32.48	9.95
Wealthy	sprayed	10.71	90.86	34.80	8.82
York	check	9.44	68.84	35.85	9.46
York	sprayed	6.88	52.92	39.35	8.36

As shown in Table 2, the Wealthy leaves had a greater R value, longer palisade cells, wider upper palisade cells, and fewer upper palisade cells in an equal area than the York variety. Within each variety, the sprayed leaves showed a lower R value, shorter and narrower palisade cells, with a greater number of cells in the first palisade layer per given area than the unsprayed leaves. These values were derived from the averages of a total of 430 pages of camera lucida drawings from 43 trees.

An analysis of variance of the ratio of the internally exposed surface to the exterior surface "R" was made. Individual tree variation was compared with variation due to varities and treatments, to determine whether varieties differed, and whether spray residues had an effect. This analysis of variance is presented in Table 3.

Table 3. Analysis of variance of R values. Trees compared with varieties and treatments.

Sources of variation	Variance			F values		
	df	Sum of sq.	Mean sq.	Data	5%	1%
Between varieties	1	158.36	158.36	120.89**	4.09	7.33
" treatments	1	69.01	69.01	52.68**		
" trees	39	51.12	1.31			
Interaction	1	5.15	5.15			
Total	42	283.64				

**Highly significant.

The variability or mean square between varieties was highly significantly greater than the variability between trees, and so one may justly assume that the mean differences found were due to varietal variation instead of tree variation. When considering treatments, the variability between treatments was highly significantly greater than the variability between trees also, indicating that the mean differences found were due to treatment variation rather than tree variation, the check trees having a highly significantly greater R value than the sprayed trees.

An analysis of variance of the total depth of the palisade layers "P" was set up, trees compared with varieties and treatments, to determine whether varieties differed in length of palisade cells, and whether spray residues had an effect on their length. This analysis of variance is presented in Table 4.

Table 4. Analysis of variance of P values. Trees compared with varieties and treatments.

Sources of variation	df	Sum of sq.	Mean sq.	F values: Data	5%	1%
Between varieties	1	14,246.27	14,246.27	273.60**	4.09	7.33
" treatments	1	2,066.19	2,066.19	39.68**		
" trees	39	2,030.74	52.07			
Interaction	1	281.79	281.79			
Total	42	18,624.99				

**Highly significant.

According to Table 4 the variability between varieties was highly significantly greater than the variability between trees, and so one may assume that the mean differences found were due to varietal variation instead of tree variation. When taking treatments into consideration, the variability between treatments was highly significantly greater than the variability between trees also, indicating that the mean differences found were due to treatment variation rather than tree variation, the check trees having palisade cells that were highly significantly longer than the sprayed trees.

An analysis of variance of the number of upper palisade cells per 2500 square microns of leaf "N" was set up, trees compared with varieties and treatments, in order to determine whether varieties differed in the number of cells per unit area, and whether spray residues had an effect on thia number. This analysis of variance ia presented in Table 5.

Table 5. Analysis of variance of N values. Trees compared with varieties and treatments.

Sources of variation	df	Variance: Sum of sq.	Mean sq.	F values: Data	5%	1%
Between varieties	1	161.60	161.60	58.13**	4.09	7.33
" treatments	1	84.14	84.14	30.27**		
" trees	39	108.43	2.78			
Interaction	1	19.41	19.41			
Total	42	373.58				

**Highly significant.

According to Table 5 the variability between varieties was highly significantly greater than the variability between trees, so one may safely assume that the mean differences found were due to varietal variation rather than tree variation. The variability between treatments was highly significantly greater than the variability between trees also, hence indicating that the mean differences found were due to treatment variation rather than tree variation, the sprayed trees having a larger number of upper palisade cells per unit area than the check trees.

An analysis of variance of the diameter of the upper layer of palisade cells "D" was set up, trees compared with varieties and treatments, to determine whether varieties differed in diameter of palisade cells, and whether spray residues had any effect on their diameter. This analysis of variance is presented in Table 6.

Table 6. Analysis of variance of D values. Trees compared with varieties and treatments.

Sources of variation	Variance			F values		
	df	Sum of sq.	Mean sq.	Data	5%	1%
Between varieties	1	2.11	2.11	19.18**	4.09	7.33
" treatments	1	13.29	13.29	120.82**		
" trees	39	4.26	.11			
Interaction	1	.20	.20			
Total	42	19.86				

**Highly significant.

As shown in Table 6, the variability between varieties was highly significantly greater than the variability between trees, indicating that the mean differences found were due to varietal variation instead of tree variation. The variability between treatments was highly significantly greater than the variability between trees also, and so one may justly assume that the mean differences found were due to treatment variation rather than tree variation, the sprayed trees having upper palisade cells that were significantly less in diameter than the check trees.

Covariance, correlation, and regression between the total depth of palisade and the R values for greenhouse leaves are presented in Table 7, to determine whether a short cut may be found for determining the R values from the depth of the palisade.

Table 7. Regression and correlation data on depth of palisade and R values.

Variety	Treatment	df	Sums of squares and products			Correlation	Regression
			Sx^2	Sxy	Sy^2	r	b
Wealthy	Check	9	17.90	81.41	497.09	.863**	4.548
Wealthy	Sprayed	9	6.03	39.76	580.09	.672*	6.594
York	Check	9	14.78	95.32	643.15	.978**	6.449
York	Sprayed	8	2.50	22.39	310.45	.804**	8.956

*Significant.
**Highly significant.

The total depth of palisade found in Table 7 was calculated by adding the length of palisade cells of the tree layers and calculating the average for each leaf. According to Table 7 there is a correlation of high significance between the total depth of palisade and the R values for all determinations except the sprayed Wealthy leaves.

Field Leaves

As was stated under materials and methods, measurements were taken from leaves of Wealthy, Jonathan, and York trees planted outdoors. The values R, P, N, and D are recorded in Table 8. Descriptions of these symbols are on page 27.

Table 8. Average R, P, N, and D for outdoor grown leaves, 1940.

Variety	Treatment	R	P in microns	N	D in microns
Wealthy	check	15.30	117.08	31.57	10.24
Wealthy	sprayed	11.96	103.64	35.93	8.20
Jonathan	check	13.54	99.60	35.72	9.34
Jonathan	sprayed	10.26	83.29	37.52	7.81
York	check	11.63	83.35	36.78	9.64
York	sprayed	7.71	60.93	37.06	8.25

According to Table 8, the Wealthy had a greater R, longer and wider palisade cells, with fewer upper palisade cells per given area than the York variety. The Jonathan were intermediate in R, P, and N, but the cells were slightly less in diameter than in the York. Within each variety and without exception, the sprayed trees showed a lower R value; the palisade cells were less in width and length, and greater in number per equal area than the check trees. These values were based on measurements from 260 pages of camera lucida drawings from 26 trees.

An analysis of variance of the ratio of the internally exposed surface to the exterior surface "R" was set up, trees compared with varieties and treatments, to determine whether varieties differed, and whether spray residues had an effect. This analysis of variance is presented in Table 9.

Table 9. Analysis of variance of R values. Trees compared with varieties and treatments.

Sources of variation	df	Variance Sum of sq.	Mean sq.	F values Data	5%	1%
Between varieties	2	62.41	31.21	17.94**	3.49	5.85
" treatments	1	81.39	89.39	46.20**	4.35	8.10
" trees	20	34.74	1.74			
Interaction	2	.59	.30			
Total	25	179.13				

**Highly significant.

According to Table 9 the variability between varieties was highly significantly greater than the variability between trees, and so one may justly assume that the mean differences found were due to varietal variation rather than tree variation. When considering treatments, the variability between treatments was highly significantly greater than the variability between trees, indicating that the mean differences found were due to treatment variation rather than tree variation, the check trees having a highly significantly greater R value than the sprayed trees.

An analysis of variance of the total depth of the palisade layers "P" was set up, trees compared with varieties and treatments, to determine whether varieties differed in length of palisade cells, and whether spray residues had an effect on their length. This analysis of variance is presented in Table 10.

Table 10. Analysis of variance of P values. Trees compared with varieties and treatments.

Sources of variation	df	Variance Sum of sq.	Mean sq.	F values Data	5%	1%
Between varieties	2	5,629.01	2,814.51	27.05**	3.49	5.85
" treatments	1	2,105.46	2,105.46	20.24**	4.35	8.10
" trees	20	2,080.48	104.03			
Interaction	2	87.02	43.51			
Total	25	9,901.97				

**Highly significant.

As shown in Table 10, the variability between varieties was highly significantly greater than the variability between trees, and so one may assume that the mean differences found were due to varietal variation instead of tree variation. The variability between treatments was also highly significantly greater than the variability between trees, indicating that the mean differences found were due to treatment variation rather than tree variation, the unsprayed trees having palisade cells that were highly significantly longer than the sprayed trees.

An analysis of variance of the number of upper palisade cells per 2500 square microns of leaf "N" was set up, trees compared varieties and treatments, in order to determine whether varieties differed in the number of cells per unit area, and whether spray residues had an effect on this number. This analysis of variance is presented in Table 11.

Table 11. Analysis of variance of N values. Trees compared with varieties and treatments.

Sources of variation	Variance			F values		
	df	Sum of sq.	Mean sq.	Data	5%	1%
Between varieties	2	42.55	21.26	5.06*	3.49	5.85
" treatments	1	21.24	21.24	5.06*	4.35	8.10
" trees	20	83.98	4.20			
Interaction	2	15.64	7.82			
Total	25	163.41				

*Significant.

According to Table 11 the variability between varieties was significantly greater than the variability between trees, so one may safely assume that the mean differences found were due to varietal variation rather than tree variation. The variability between treatments was also significantly greater than the variability between trees indicating that the mean differences found were due to treatment variation rather than tree variation, the sprayed trees having a significantly larger number of upper palisade cells per unit area than the check trees.

An analysis of variance of the diameter of the upper layer of palisade cells "D" was set up, trees compared with varieties and treatments, to determine whether varieties differed in diameter of palisade cells, and whether spray residues had an effect. This analysis of variance is presented in Table 12.

Table 12. Analysis of variance of D values. Trees compared with varieties and treatments.

Sources of variation	Variance			F values		
	df	Sum of sq.	Mean sq.	Data	5%	1%
Between varieties	2	1.66	.83	2.18	3.49	5.85
" treatments	1	16.57	16.57	44.61**	4.35	8.10
" trees	20	7.58	.38			
Interaction	2	.41	.21			
Total	25	26.22				

**Highly significant.

According to Table 12, there was no significant difference between the variability between varieties and the variability between trees. However, the variability between treatments was highly significantly greater than the variability between trees, and one may thus justly assume that the mean differences found were due to treatment variation instead of tree variation, the sprayed leaves having upper palisade cells that were highly significantly less in diameter than the unsprayed leaves.

Covariance, correlation, and regression between the total depth of palisade and the R values for field leaves are presented in Table 13 to determine whether a short cut may be found for determining the R values from the depth of the palisade.

Table 13. Regression and correlation data on depth of palisade and R values.

Variety	Treatment	df	Sums of squares and products			Correlation	Regression
			Sx^2	Sxy	Sy^2	r	b
Wealthy	check	1	2.80	16.06	101.34	.954	5.7357
Wealthy	sprayed	1	11.90	107.48	971.38	.9997**	9.0319
Jonathan	check	3	7.29	68.07	660.27	.981**	9.3374
Jonathan	sprayed	3	2.85	22.17	196.84	.936*	7.7789
York	check	3	0.70	6.10	77.88	.827	8.7143
York	sprayed	3	0.39	2.35	72.79	.442	6.0256

*Significant.
**Highly significant.

Calculations for Table 13 were carried out by the same method as for Table 7. In Table 13 the degrees of freedom were so few that correlations had to be extremely high to be significant. The correlation for York sprayed was extremely low. However, the regression values were not significantly different, but were so far apart that they could not be pooled for very accurate calculations.

Covariance, correlation, and regression between the total depth of palisade and the R values for all varieties, treatments, and conditions are presented in Table 14, to determine whether a short cut may be found for determining the R values from the depth of the palisade.

Table 14. Pooled regression and correlation data on depth of palisade and R values.

df	Sum of squares and products			Correlation coefficient	Regression coefficient
	Sx^2	Sxy	Sy^2	r	b
67	469.67	3562.23	29,696.28	.954**	7.58

**Highly significant

The correlation for the whole group is very highly significant. Using this correlation, the ratio of the internally exposed surface to the external surface may be computed from the total depth of palisade mesophyll (Fig. 1).

Sx^2 equals the variance of the ratio of the internally exposed surface to the external surface, Sy^2 equals the variance of the total depth of palisade layers, and Sxy equals covariance of X and Y.

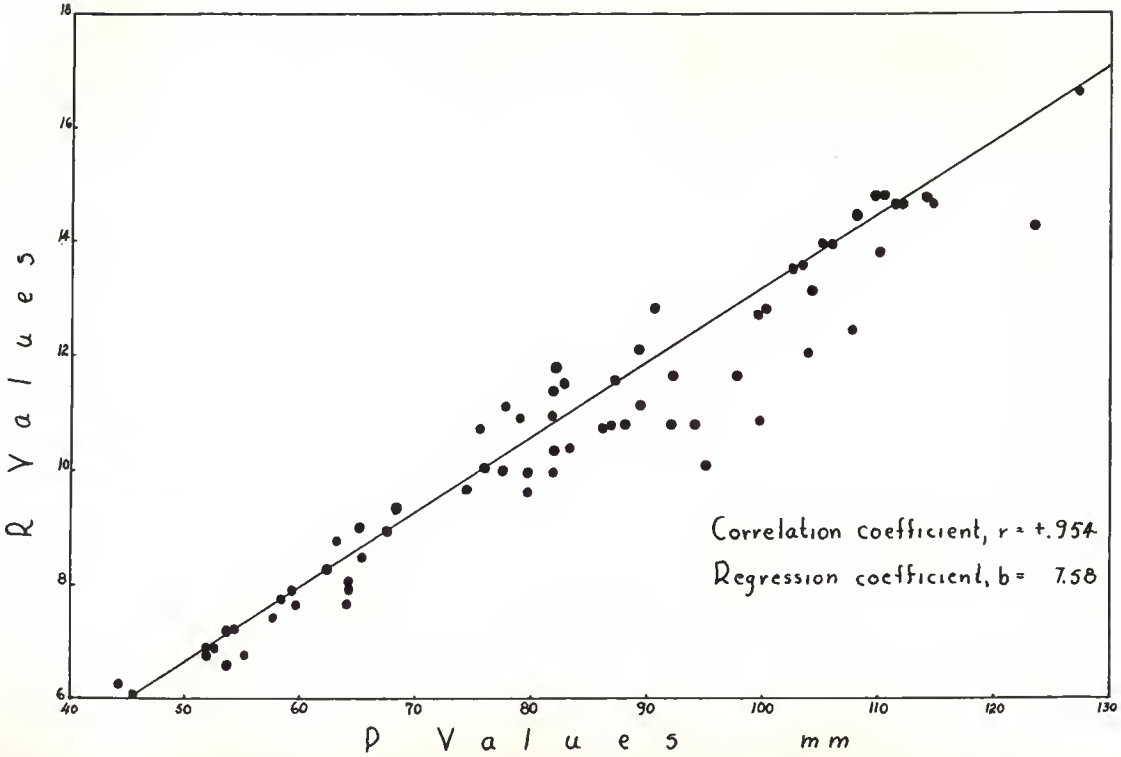

Fig 1. Regression Line – Sprayed and Unsprayed, Field and Greenhouse Leaves

The midseason group of leaves, collected July 1, 1940, after the last lead arsenate spray, were collected and used as a check on accuracy of sampling and also to determine whether the spray materials had an accumulative effect as the season progressed. For this group of leaves, treated in the same manner as the end of season field leaves, only the palisade lengths were measured. The means of their total depth of palisade are compared with the corresponding value of the leaves from the same trees at the end of the season in Table 15.

Table 15. Means of total depth of palisade, measured in microns, for field leaves at midseason and at end of season.

Variety	Treatment	Midseason	End of season
Wealthy	check	111.27	117.08
Wealthy	sprayed	102.32	103.64
Jonathan	check	107.11	99.60
Jonathan	sprayed	78.37	83.29
York	check	82.49	83.35
York	sprayed	60.08	60.93

According to Table 15, the differences between check and sprayed trees in a variety were approximately the same at midseason as they were at the end of the season. The Wealthy check was a little higher at the end of the season, but there was a greater difference in the Jonathan at midseason. The York values were practically identical for both periods.

A comparison of the means of the Wealthy and York varieties in the greenhouse and in the field is presented in Table 16.

Table 16. Mean R values for both varieties and treatments in the greenhouse and in the field.

Variety	Treatment	Location	R	P in microns	N	D in microns
Wealthy	check	field	15.30	117.08	31.57	10.24
Wealthy	check	greenhouse	13.40	104.49	32.48	9.95
Wealthy	sprayed	field	11.96	103.64	35.98	8.20
Wealthy	sprayed	greenhouse	10.71	90.86	34.80	8.82
York	check	field	11.63	83.35	36.78	9.64
York	check	greenhouse	9.44	68.84	35.85	9.46
York	sprayed	field	7.71	60.93	37.06	8.25
York	sprayed	greenhouse	6.88	52.92	39.35	8.36

According to Table 16, the R value was greater and the total depth of palisade layers was greater for any variety and treatment in the field than for the corresponding variety and treatment in the greenhouse. The number of cells of the upper palisade per unit area and their diameters showed no great differences as to location.

DISCUSSION

An analysis of the data collected in this investigation was undertaken in an effort to determine (1) whether the leaves of the varieties used differed in their R values, (2) whether spray residues had an effect on the internal structure, and (3) whether some short cut for calculating the R value could be found.

Tables 2 and 8 include the means of the ratios of the internally exposed surface to the exterior surface, "R"; the total depth of the palisade layers, "P"; the average number of upper palisade cells per 2500 square microns of leaf area, "N"; and the average diameter of the first layer of palisade cells, "D". These tables indicate that under both greenhouse and field conditions, with both sprayed and unsprayed leaves, the Wealthy foliage has a greater R value, greater total depth of palisade layers, wider cells in the first palisade, and a smaller number of first palisade cells per unit area than the York variety. The field grown Jonathan leaves were intermediate. Within each variety and for both locations, the sprayed leaves have a lower R value, shorter palisade cells, narrower first layer palisade cells, with a greater number in the first palisade layer per unit of leaf area than the unsprayed leaves.

Tables 3 and 9 contain data on the analysis of variance

of R values with trees in a variety compared with varieties and treatments. These data show that the variability between varieties was highly significantly greater than the variability between trees for both greenhouse and field grown leaves. It may be assumed, therefore, that the mean differences found were due to varietal variation between varieties rather than to variation within the variety. When considering treatments, the variability between treatments was highly significantly greater than the variability between trees for both locations also, indicating that the mean differences found were due to treatment variation rather than tree variation, the check trees having a highly significantly greater R value than the sprayed trees. It may thus be concluded from these data that the varieties studied have R values whose differences are highly significant, and that spray residues will reduce these R values highly significantly.

Tables 4 and 10 contain the data on the analysis of variance of the total depth of palisade layers, "P", with trees against varieties and treatments. These data indicate that the variability between varieties was highly significantly greater than the variability between trees within the variety for both greenhouse and field leaves. This leads to the conclusion that the mean differences found were due to varietal variation rather than tree variation. The variability between treatments was highly significantly greater

than the variability between trees, indicating that the mean differences found were due to treatment variation rather than tree variation, the sprayed trees in both locations having a total depth of palisade layers which was highly significantly less than that of the check trees. These data show that the varieties studied have highly significantly different total depth of palisade layers, and that spray residues will reduce this total depth highly significantly.

Tables 5 and 11 include the data for the analysis of variance of the number of upper palisade cells per 2500 square microns of leaf surface, "N", trees compared with varieties and treatments. These data show that the variability between varieties was highly significantly greater than the variability between trees for the greenhouse trees, and significantly greater for the field leaves, and so one may justly assume that the mean differences found were due to varietal variation rather than to variation within the variety. The variability between treatments was also highly significantly greater than the variability between trees for the greenhouse trees and significantly greater for the field trees, indicating that the mean differences found were due to treatment variation rather than tree variation, the sprayed trees having a larger number of upper palisade cells per unit area than the check trees. It may, therefore, be concluded from these data that the varieties studied have a difference in the number of upper palisade cells per unit area, and that this difference is

significant and spray residues will increase this number enough to be significant.

Tables 6 and 12 contain the data for the analysis of variance of the diameters in microns of the upper palisade cells, "D", trees compared with varieties and treatments. These data show that the variability between varieties was highly significantly greater than the variability between trees for the greenhouse trees, but no significant difference for the field leaves, indicating that for the greenhouse leaves the mean differences found were due to varietal variation rather than tree variation, while tree variation was responsible for the differences in field leaves. The variability between treatments was highly significantly greater than the variability between trees for both locations, so one may conclude that the mean differences found were due to treatment variation rather than tree variation, the sprayed trees having upper palisade cells with smaller diameters than the check trees. These data indicate that the varieties studied have a highly significantly different width of upper palisade in the greenhouse, but no significance in the field, and that spray residues will cause a highly significant decrease in this diameter in both locations.

Since the palisade mesophyll contained about 85 per cent of the total internally exposed surface (Kenworthy, 1939) and substantiated by general observations in this investigation, it would be assumed that any measurement having a high

correlation with R would probably come from this area. From analysis of variance and other data it appeared that the total depth of palisade layers varied positively and very closely with R. The total depth of palisade layers was considered for this relationship as this would also eliminate the necessity of making tangential slides.

This analysis by covariance for correlation and regression between the total depth of palisade layers and the R values was carried out separately for the greenhouse and field leaves in Tables 7 and 13, with each variety, treatment, and location determined separately. The degrees of freedom of number of samples were so few in each case that correlations had to be extremely high to be significant and regressions could vary a great deal and still not be significantly different. It was therefore decided to pool the lot, using the means for the entire group, including all varieties, treatments, and locations.

Covariance, correlation, and regression between the total depth of palisade and the R values for all varieties, treatments, and locations were presented in Table 14, to determine whether a short cut could be found for determining the R values from the depth of the palisade. The correlation for the whole group is very highly significant. Using this correlation, the ratio of the internally exposed surface to the external surface may be computed directly from the total depth of palisade mesophyll. Due to the variation of cell

length in the palisade mesophyll, the measurement of each layer should be made separately and then totaled instead of measuring the total depth of the palisade mesophyll in one measurement. Since the regression coefficients are fairly close, one regression line may be used to compute the ratio of the internally exposed surface to the external surface for all varieties for rough or preliminary work, but an individual regression for each condition would be more desirable for accurate results.

There was greater variation in the total depth of palisade layers than in the ratio of the internally exposed surface to the external surface because the values of the total depth of palisade layers were numerically greater than the R values.

All of the varieties were found to have three layers of palisade cells. The third layer was frequently only partially developed or entirely missing in the York variety, and in a few of the Wealthy cross section slides there was a fourth partial layer. When the third layer was missing or less than half developed, or only a few cells were present, it was omitted in the calculations. The fourth layer was termed "palisade like" spongy mesophyll and was measured with the spongy mesophyll. In a few cross section slides a layer of cells that resembled palisade cells was observed just above the lower epidermis. These cells were also measured as spongy mesophyll cells, and were probably developed near the lower

epidermis due to high light intensity on the lower surface of the leaf (Bergen, 1904).

Variations between varieties and treatments growing in the field may be influenced somewhat by environmental factors, but analysis of variance shows that certainly the greater part of the variation was due to varietal differences and differences due to treatment. In the greenhouse where all factors other than tree and varietal variations were controlled, the results were practically identical with those in the field.

A group of leaves were collected from the field trees during midseason and used as a check on accuracy of sampling and to determine whether the spray materials had an accumulative effect as the season progressed. For this group of leaves, treated in the same manner as the end-of-season field leaves, only the palisade lengths were measured. The means of their total depth of palisade are compared with the corresponding values of the leaves from the same trees at the end of the season in Table 15. According to this table, the differences between sprayed and check trees were as marked at midseason as at the end of the season, indicating that as soon as the leaves were full grown the sprayed leaves had been reduced in size as much as they would be from continued applications throughout the remainder of the season. The individual trees within each variety and treatment ranked in the same order as to depth of palisade layers for both poriods, which would indicate that the leaf sampling was extensive enough to

be highly representative.

A comparison of the means of the Wealthy and York varieties in the greenhouse and in the field was presented in Table 16. These data show that the R value and the total depth of palisade layers was greater for any variety and treatment in the field than for the corresponding variety and treatment in the greenhouse. These results are expected and in line with literature on the subject, for light has been considered by a number of workers to have the greatest influence on the anatomy of leaves. The intensity of light in the greenhouse is much less than that in the field.

Most of the workers who have studied the photosynthetic behavior of leaves and its relation to leaf anatomy have used the structure of the spongy mesophyll as an index to the internal leaf structure, and this spongy mesophyll has been studied as the part of the leaf having the greatest influence on photosynthesis. According to Haberlandt (1928), photosynthesis is a subsidiary function of the spongy mesophyll. This statement has a great deal of evidence to back it up. In a leaf that is perpendicular to the incident rays of light, the first palisade region would have the greatest intensity of light, with the intensity being greatly reduced as it passes through the leaf toward the spongy mesophyll. That the spongy mesophyll has a low photosynthetic activity is again indicated by the fact that it contains but a small percentage of the chloroplasts of a leaf (Haberlandt, 1928),

often as low as 11 per cent.

If we assume that the rate of photosynthesis is related to the extent of the internally exposed surface, the palisade mesophyll of apple leaves would have a greater photosynthetic activity than the spongy mesophyll, and the photosynthetic activity of a leaf region would decrease as the region lies more distant from the upper epidermis, for the per cent of internally exposed surface decreases from the first layer of palisade to the lower epidermis.

When the size of chloroplasts was considered as an index to photosynthetic activity, the chloroplasts were observed to be larger in the greenhouse grown leaves than in the field grown leaves. The decrease in the intensity of light may be considered as the cause of this. According to Haberlandt (1928), the species he studied had larger chloroplasts in the spongy mesophyll than in the palisade mesophyll. This was also true for the apple leaves studied in this investigation. The chloroplasts also varied negatively with the diameter of the palisade cells, and a varietal variation was also in evidence. The above evidence would indicate that the size of chloroplasts would vary negatively with the rate of photosynthesis and with ratio of internal exposed surface to external surface, indicating again that internal exposed surface has an effect on rate of photosynthesis.

The majority of workers who have studied the effect of spray residues on the rate of photosynthesis of apple leaves

have shown that these spray materials severely cut down the rate of carbon dioxide assimilation. By the use of carbon dioxide absorption towers they have shown that even where there is no indication of visible injury to the leaves, the rate of photosynthesis has been greatly reduced. The results of this investigation have shown that spray residues greatly reduce the ratio of the internal exposed surface to the exterior surface, largely through shortening the palisade mesophyll cells. It may be deduced from the foregoing evidence that leaf structure has an effect on photosynthesis due to differences in extent of absorption surfaces for gas exchanges, and the conclusion may thus be drawn that spray residues reduce the rate of photosynthesis due to alteration of amount of absorption surface.

The author believes the relation of the ratio of the internal exposed surface to the external surface of apples leaves to rate of photosynthesis offers a field for much further research. The spray materials which have been found by other workers to reduce the rate of photosynthesis to the greatest degree were found in this investigation to reduce greatly the R value. Further research on a number of spray materials, including some of the less caustic, would be desirable in further studying the relationship between apray residues, photosynthetic activity, and R values. It would also be advisable to spray half of a tree and leave the other half unsprayed as a check, which would obviate individual

tree variation. As there was no material difference between midseason and end-of-season leaves with the same treatment, it would be well to spray some mature leaves which had been left unsprayed up to the time of maturity to find out whether sprays alter internal structure of fully grown leaves. The data in this work indicated that the sprays had their greatest effect on the young leaves. The correlation between total depth of palisade layers and R value deserve further study, as the estimation of R from palisade depth would reduce the time consumed in this work to a fraction of that required by the present method used. The tangential sections should always be taken from the same leaf as the cross sections for this work. It would be advisable to make complete measurements for a few leaves per tree or variety, and then make large numbers of palisade depth measurements, after having computed the ratio of palisade depth to R value from the few complete measurements.

Again assuming that the ratio of the internally exposed surface to the exterior surface is an important factor in photosynthetic activity, the lower R value of the York variety may be offered as one of the factors contributing to its biennial bearing habit in the Missouri Valley. This fact may even be suggested for determining vigor of seedling trees while still in the nursery, and thus save several years in selection.

SUMMARY AND CONCLUSIONS

1. The ratios of the internally exposed surface to the external surface for the greenhouse grown leaves of the varieties were: Wealthy check, 13.40; Wealthy sprayed, 10.71; York check, 9.44; and York sprayed, 6.88. These differences were highly significant between both varieties and treatments.

2. The ratios of the internally exposed surface to the external surface for the field grown leaves of the varieties studied were: Wealthy check, 15.30; Wealthy sprayed, 11.96; Jonathan check, 13.54; Jonathan sprayed, 10.26; York check, 11.63; and York sprayed, 7.71. These differences were highly significant between both varieties and treatments.

3. The total depth of palisade mesophyll cells, measured directly in microns, for the greenhouse grown leaves of the varieties studied were: Wealthy check, 104.49; Wealthy sprayed, 90.86; York check, 68.84; and York sprayed, 52.92. These differences were highly significant between both varieties and treatments.

4. The average total depth of palisade mesophyll cell layers, measured directly in microns, for the field grown leaves of the varieties studied were: Wealthy check, 117.08; Wealthy sprayed, 103.64; Jonathan check, 99.60; Jonathan sprayed, 83.29; York check, 83.35; and York sprayed, 60.93. These differences between both varieties and treatments were

highly significant.

5. The average number of upper palisade cells per 2500 square microns of leaf area for the greenhouse grown leaves of the varieties studied were: Wealthy check, 32.48; Wealthy sprayed, 34.80; York check, 35.85; and York sprayed, 39.35. These differences between both varieties and treatments were highly significant.

6. The average number of upper palisade cells per 2500 square microns of leaf area for the field grown leaves of the varieties studied were: Wealthy check, 31.57; Wealthy sprayed, 35.93; Jonathan check, 35.72; Jonathan sprayed, 37.52; York check, 36.78; and York sprayed, 37.06. These differences between both varieties and treatments were significant.

7. The average diameter of the first layer of palisade cells, measured in microns, for the greenhouse grown leaves of the varieties studied were: Wealthy check, 9.95; Wealthy sprayed, 8.82; York check, 9.46; and York sprayed, 8.36. These differences between both varieties and treatments were highly significant.

8. The average diameter of the first layer of palisade cells, measured in microns, for the field grown leaves of the varieties studied were: Wealthy check, 10.24; Wealthy sprayed, 8.20; Jonathan check, 9.34; Jonathan sprayed, 7.81; York check, 9.64; and York sprayed, 8.25. These values were highly significantly different between treatments within each variety, but the differences between varieties were not

significant.

9. The correlation coefficients between total depth of palisade layers and R values for the greenhouse leaves were significant, while the regression coefficients showed no significant difference.

10. The correlation coefficients between total depth of palisade layers and R values for the field leaves were high, in several cases highly significant, and the regression coefficients showed no significant difference.

11. The correlation coefficient between total depth of palisade layers and R values, by pooled covariance, assuming all varieties, treatments, and locations as one, was .954, which was highly significant. The regression coefficient was 7.58.

12. In the field grown leaves, the differences between leaves from check and leaves from sprayed trees within a variety were approximately the same at midseason as they were at the end of the season.

13. The R values and total depth of palisade layers of leaves were much greater for any variety and treatment in the field than for the corresponding variety and treatment in the greenhouse.

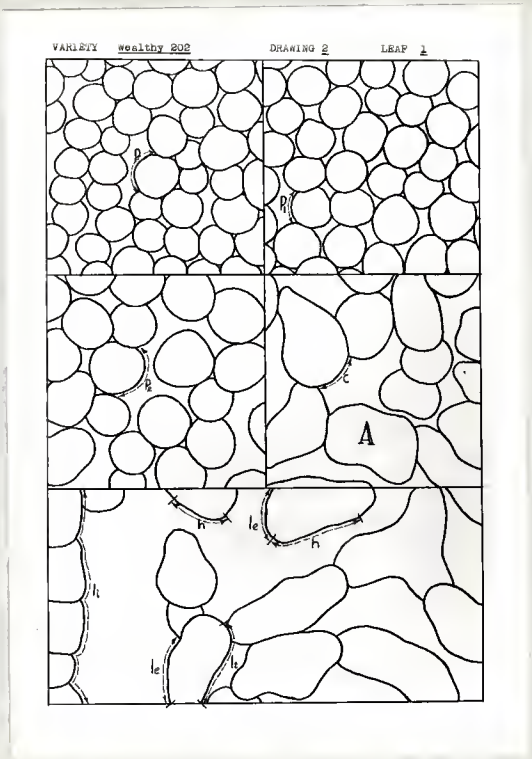

Fig. 2. Representative drawings of the Wealthy variety (x$\frac{1}{2}$). Upper left - first layer of palisade cells in tangential section. Upper right - second layer of palisade cells in tangential section. Center left - third layer of palisade cells in tangential section. Center right - spongy mesophyll in tangential section. Bottom - area 50 microns wide across spongy mesophyll in cross section. (Refer to page 27 for definition of symbols.)

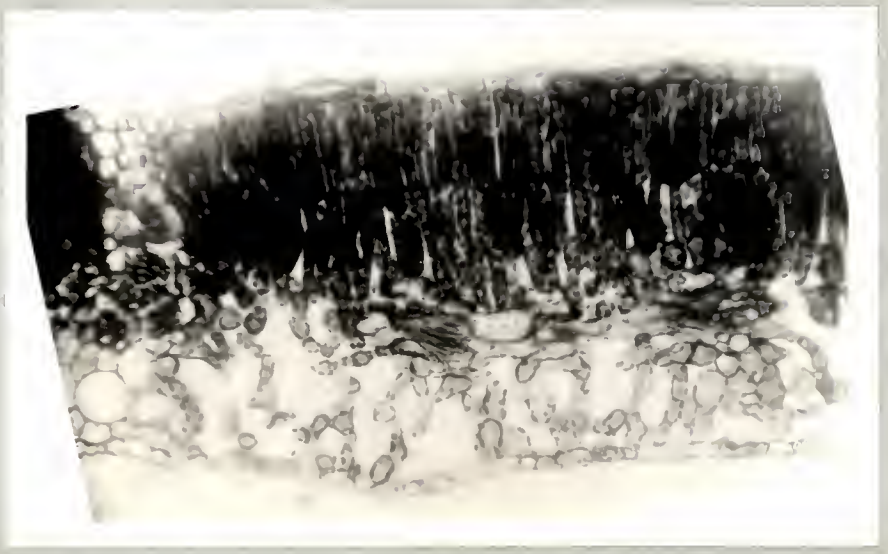

Fig. 3. Cross section of Wealthy unsprayed field leaf (x315). R value = 16.31; P value = 124.92 microns; N value = 29.

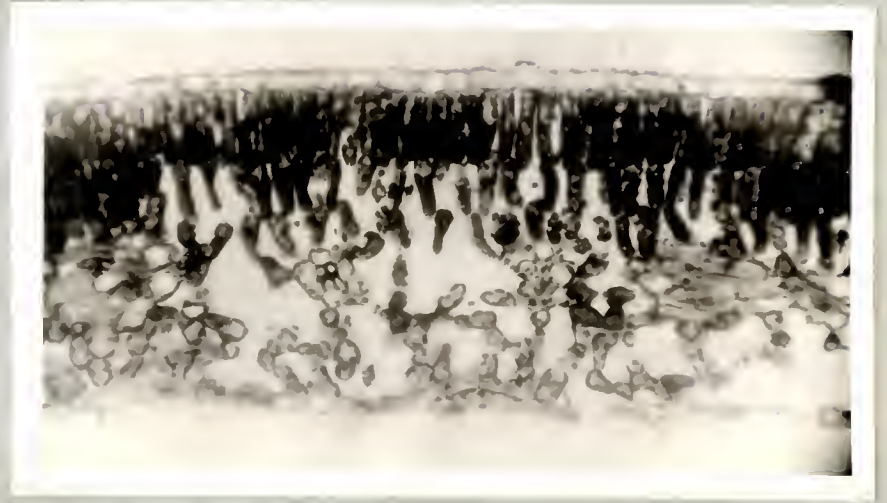

Fig. 4. Cross section of Wealthy sprayed field leaf (x315). R value = 13.82; P value = 119.64 microns; N value = 42.

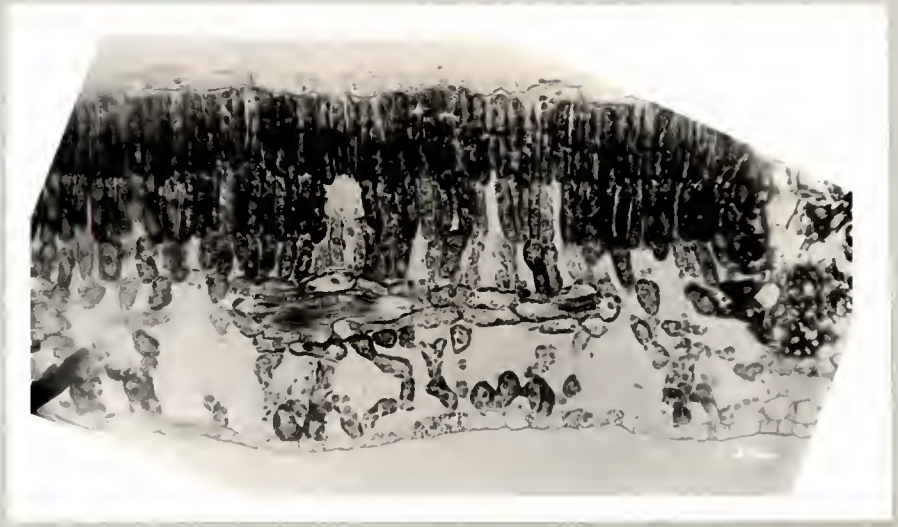

Fig. 5. Cross section of Wealthy unsprayed greenhouse leaf (x315). R value = 14.74; P value = 120.96 microns; N value = 30.

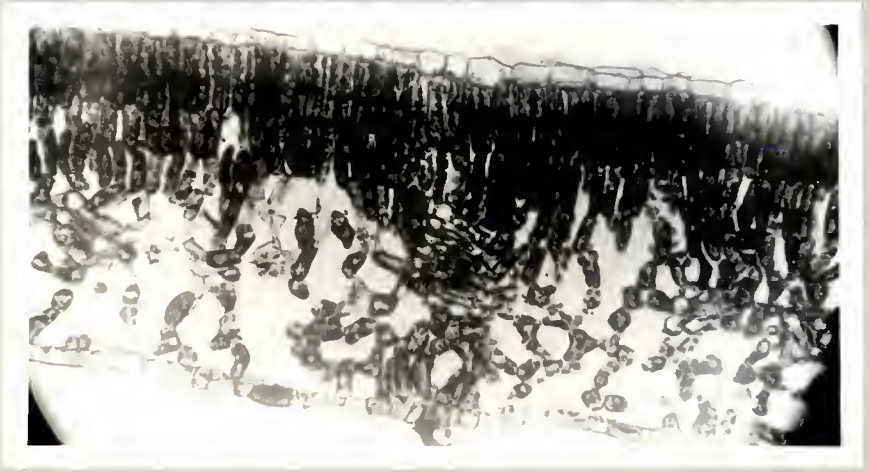

Fig. 6. Cross section of Wealthy sprayed greenhouse leaf (x315). R value = 8.81; P value = 87.84 microns; N value = 39.

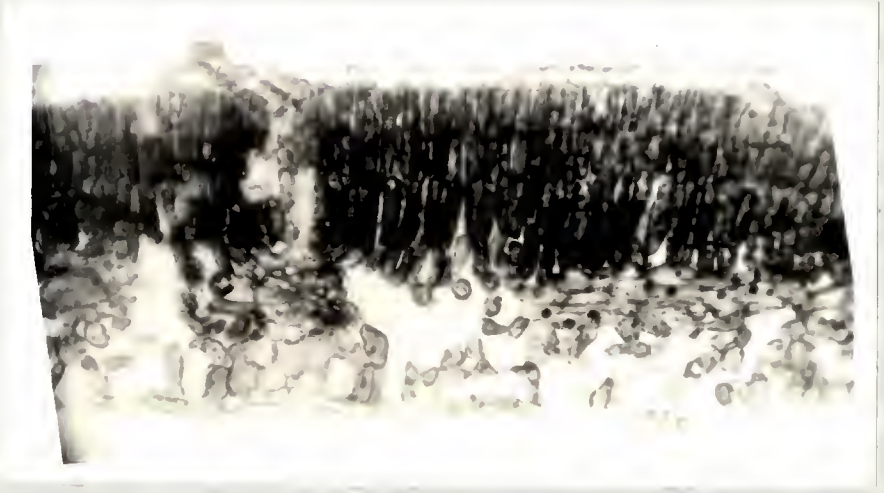

Fig. 7. Cross section of Jonathan unsprayed field leaf (x315). R value = 9.64; P value = 72.96 microns; N value = 24.

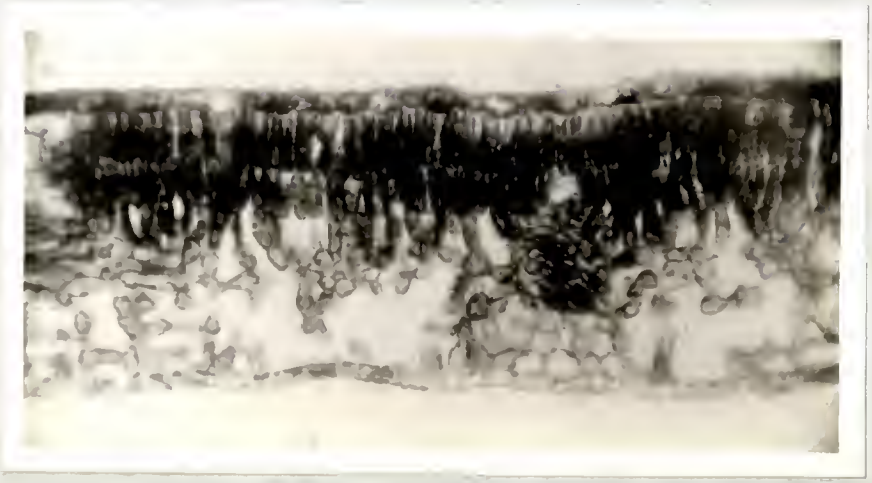

Fig. 8. Cross section of Jonathan sprayed field leaf (x315). R value = 11.96; P value = 89.52 microns; N value = 45.

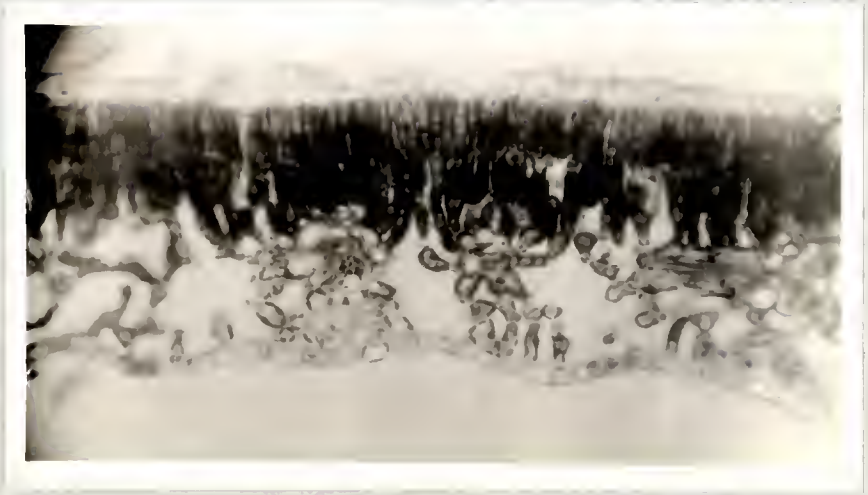

Fig. 9. Cross section of York unsprayed field leaf (x315). R value = 12.42; P value = 92.88 microns; and N value = 39.

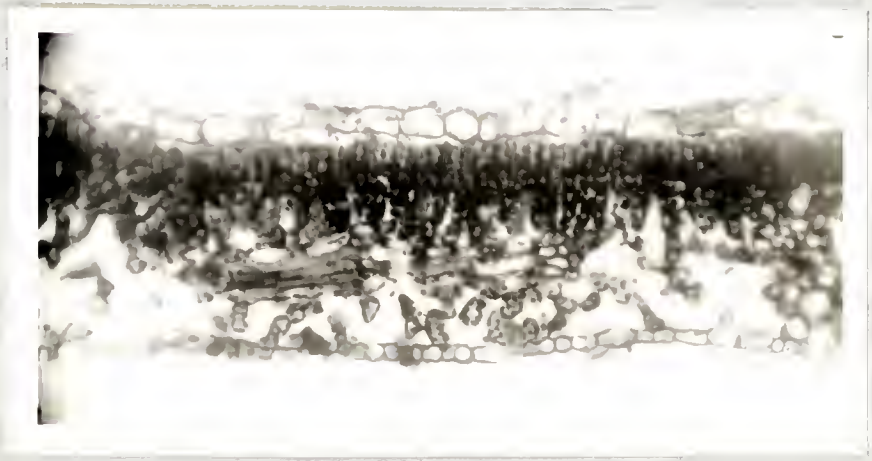

Fig. 10. Cross section of York sprayed field leaf (x315). R value = 7.12; P value = 52.68 microns; N value = 39.

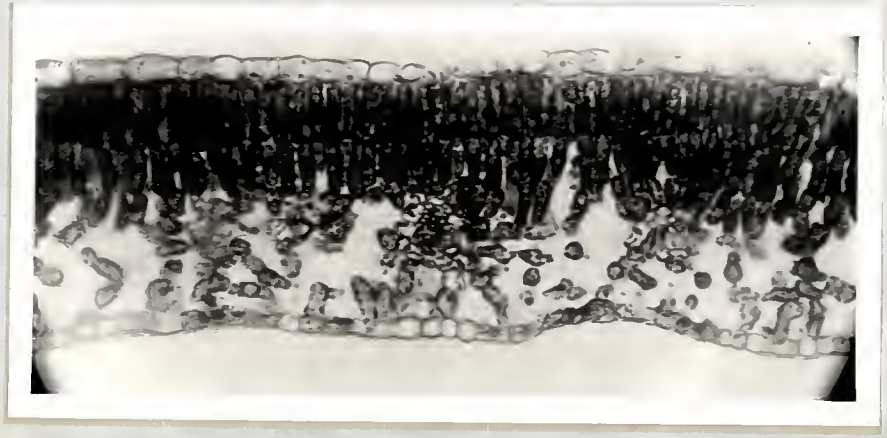

Fig. 11. Cross section of York unsprayed greenhouse leaf (x315). R value = 12.68; P value = 86.52 microns; N value = 35.

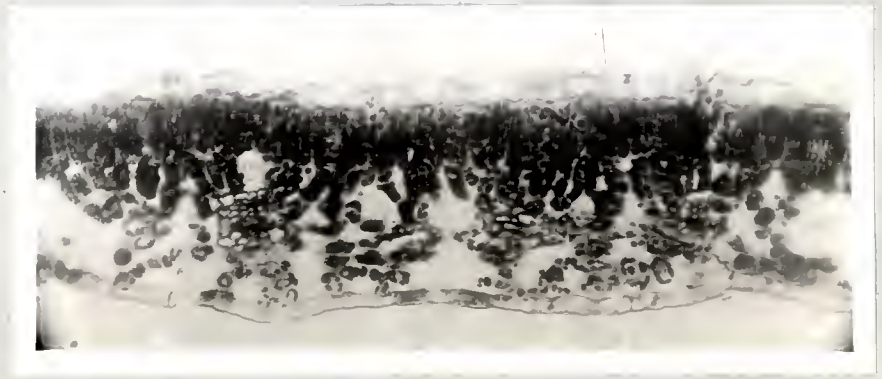

Fig. 12. Cross section of York sprayed greenhouse leaf (x315). R value = 7.05; P value = 59.76 microns; N value = 44.

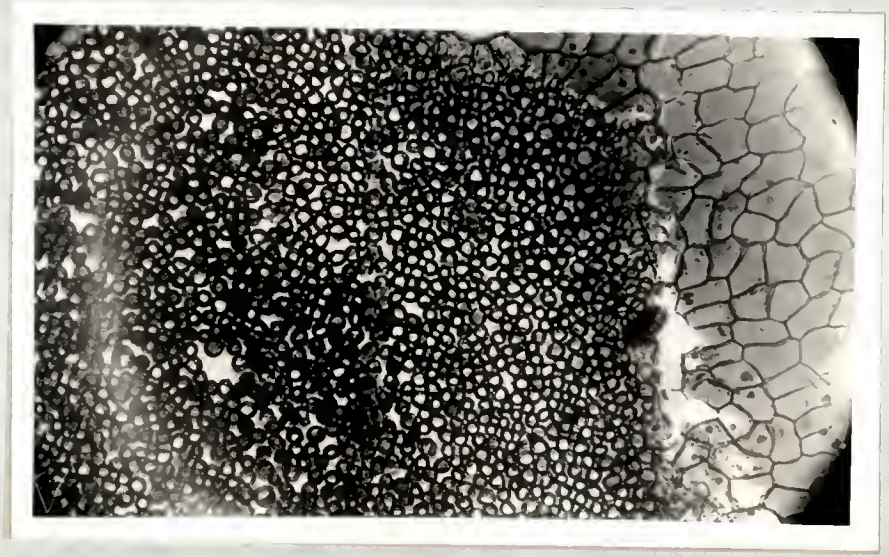

Fig. 13. Tangential section through the first layer of palisade, Wealthy unsprayed greenhouse leaf (x315). R value = 13.72; N value = 29.

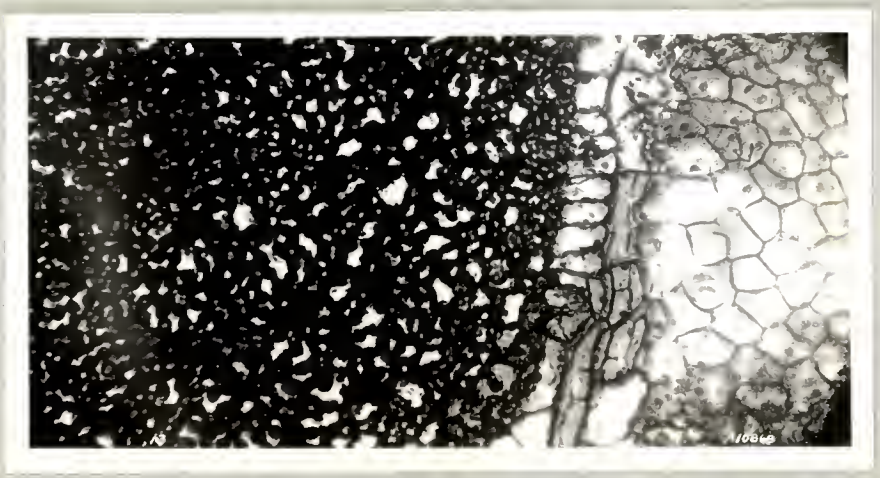

Fig. 14. Tangential section through the first layer of palisade, Wealthy sprayed greenhouse leaf (x315). R value = 11.23; N value = 29.

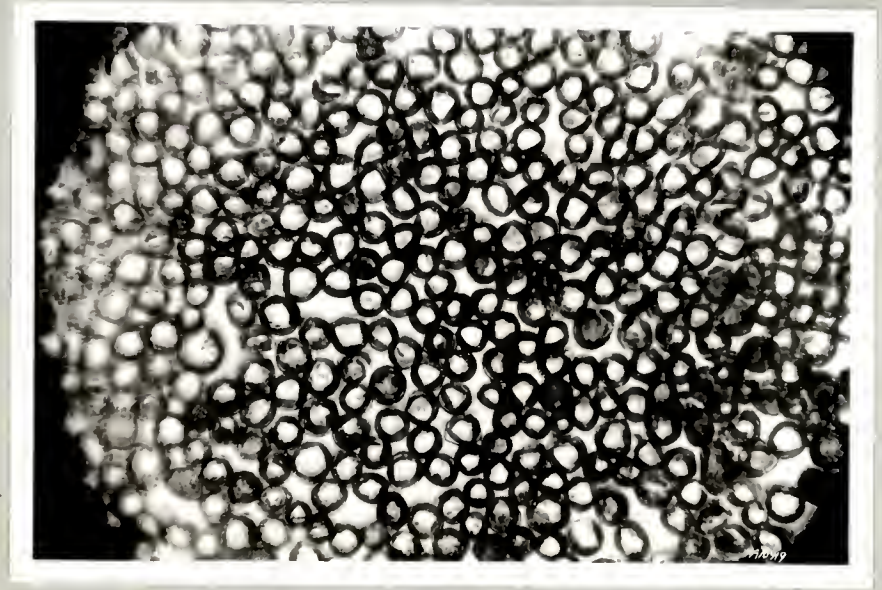

Fig. 15. Tangential section through the first layer of palisade, Wealthy unsprayed greenhouse leaf (x700). R value = 13.72; N value = 29.

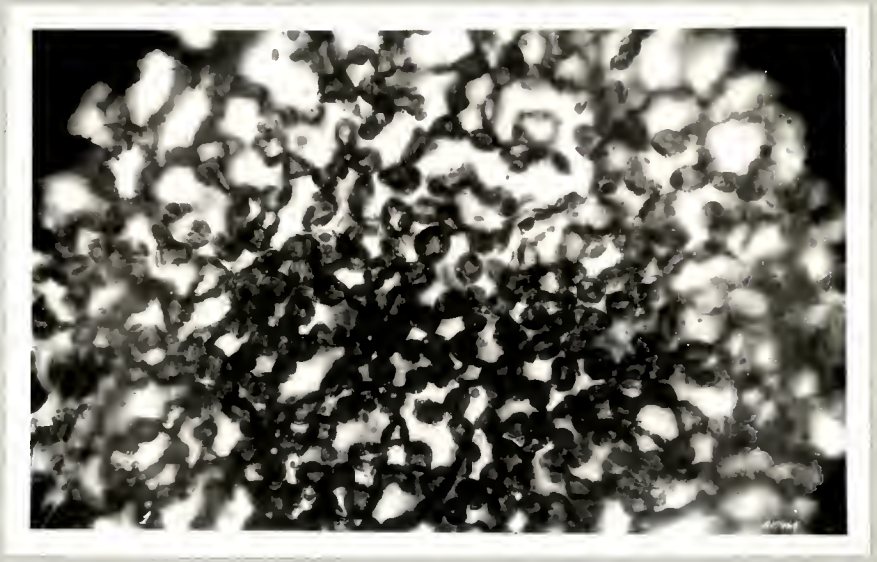

Fig. 16. Tangential section through the first layer of palisade, Wealthy sprayed greenhouse leaf (x700). R value = 11.23; N value = 29.

Fig. 17. Tangential section through the first layer of palisade, York unsprayed greenhouse leaf (x315).

Fig. 18. Tangential section through the first layer of palisade, York sprayed greenhouse leaf (x315).

Fig. 19. Tangential section through the first layer of palisade, York unsprayed greenhouse leaf (x700).

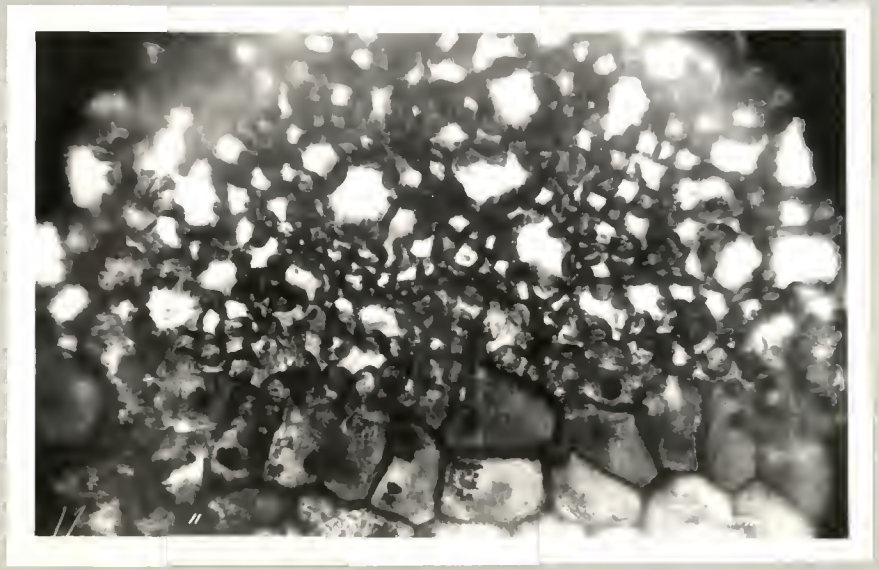

Fig. 20. Tangential section through the first layer of palisade, York sprayed greenhouse leaf (x700).

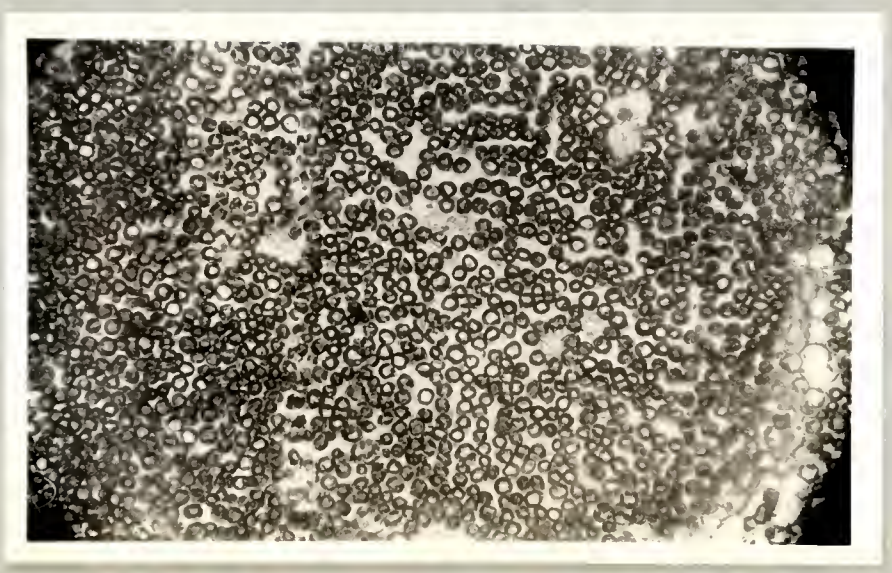

Fig. 21. Tangential section through the second layer of palisade, Wealthy unsprayed greenhouse leaf (x315).

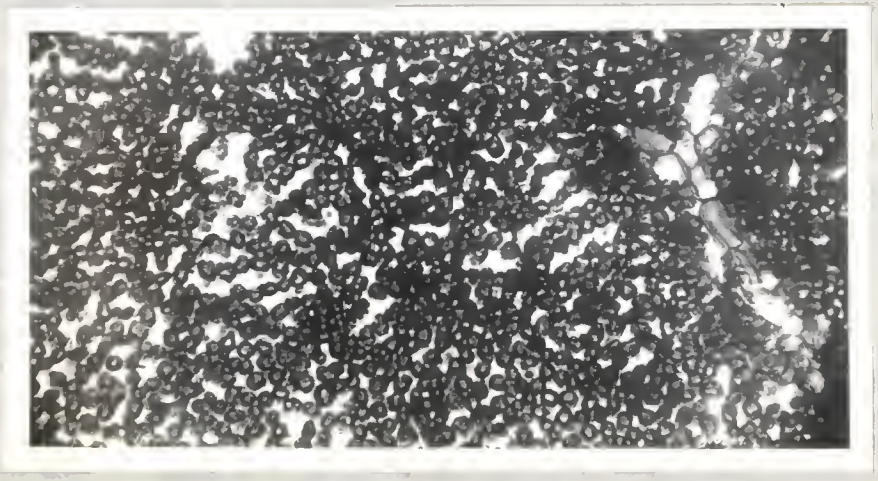

Fig. 22. Tangential section through the second layer of palisade, Wealthy sprayed greenhouse leaf (x315).

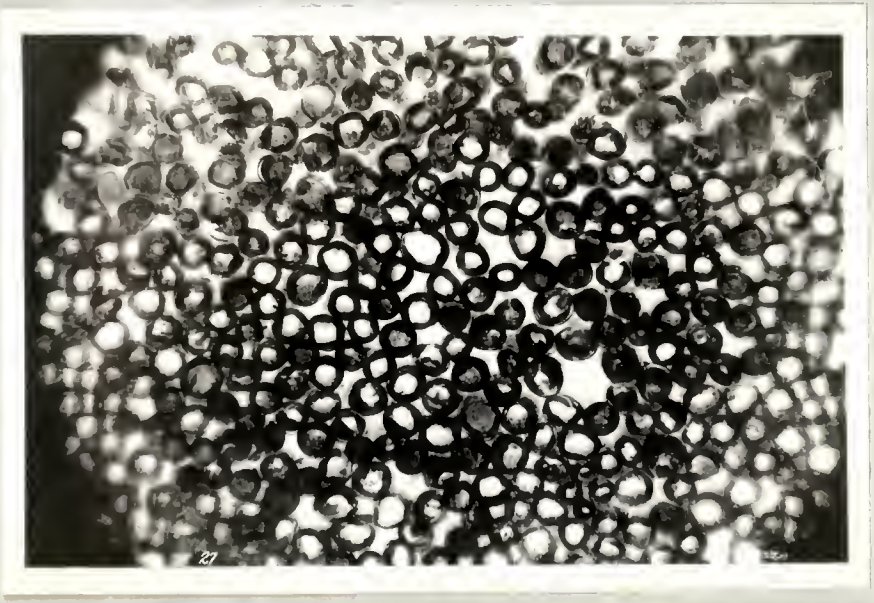

Fig. 23. Tangential section through the second layer of palisade, Wealthy unsprayed greenhouse leaf (x700).

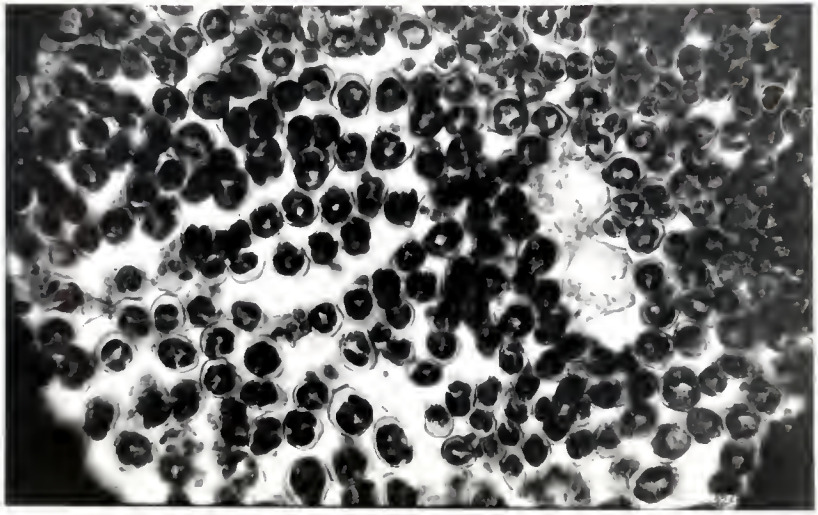

Fig. 24. Tangential section through the second layer of palisade, Wealthy sprayed greenhouse leaf (x700).

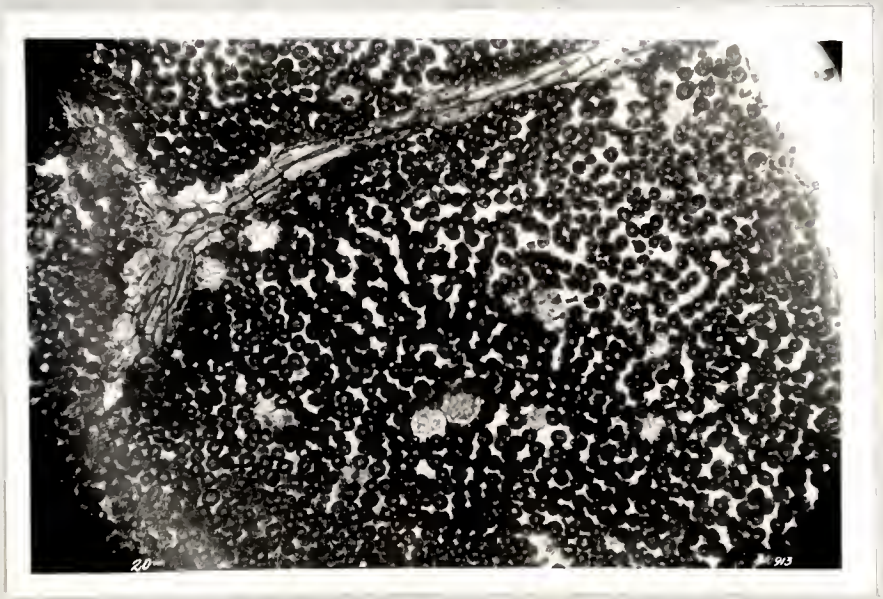

Fig. 25. Tangential section through the second layer of palisade, York unsprayed greenhouse leaf (x315).

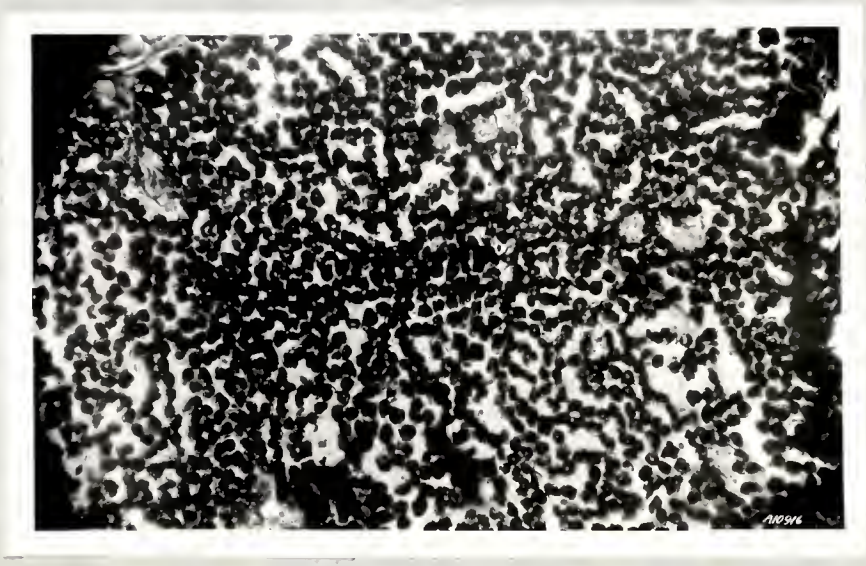

Fig. 26. Tangential section through the second layer of palisade, York sprayed greenhouse leaf (x315).

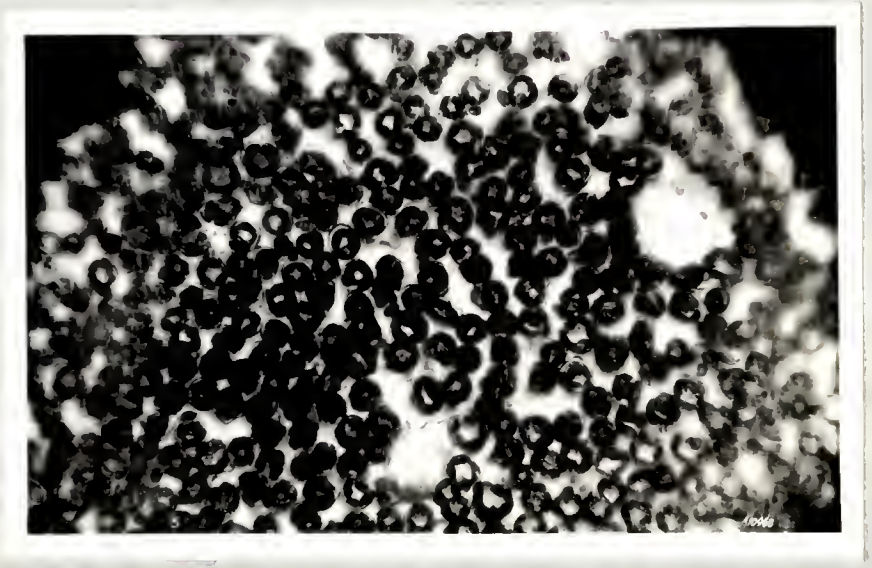

Fig. 27. Tangential section through the second layer of palisade, York unsprayed greenhouse leaf (x700).

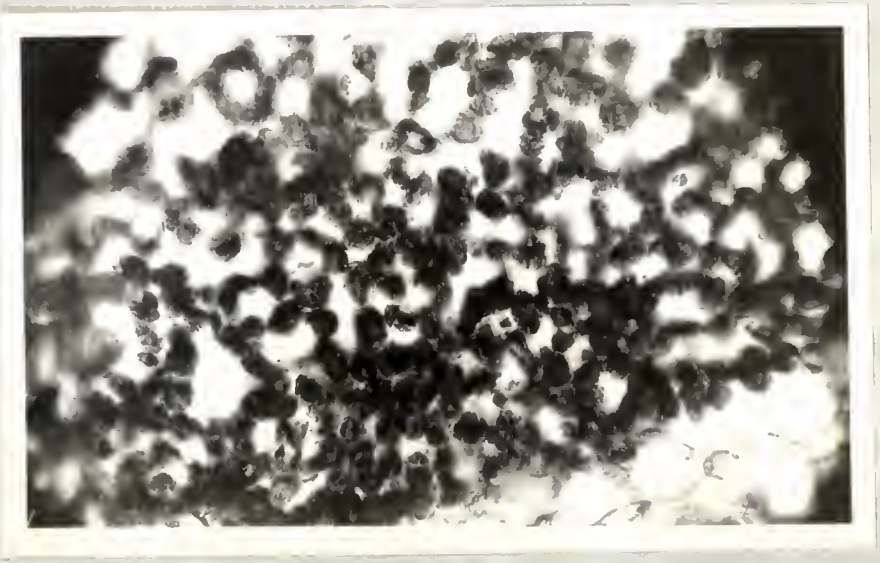

Fig. 28. Tangential section through the second layer of palisade, York sprayed greenhouse leaf (x700).

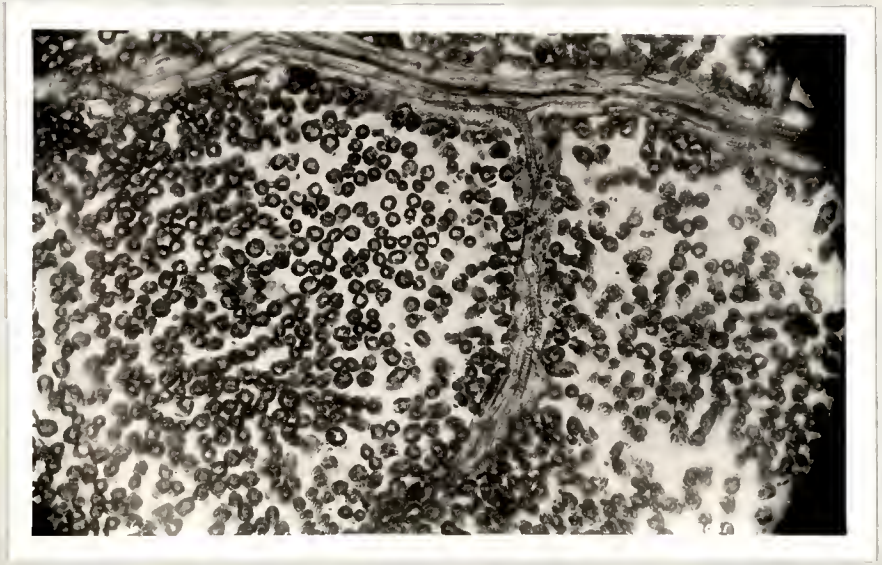

Fig. 29. Tangential section through the third layer of palisade, Wealthy unsprayed greenhouse leaf (x315).

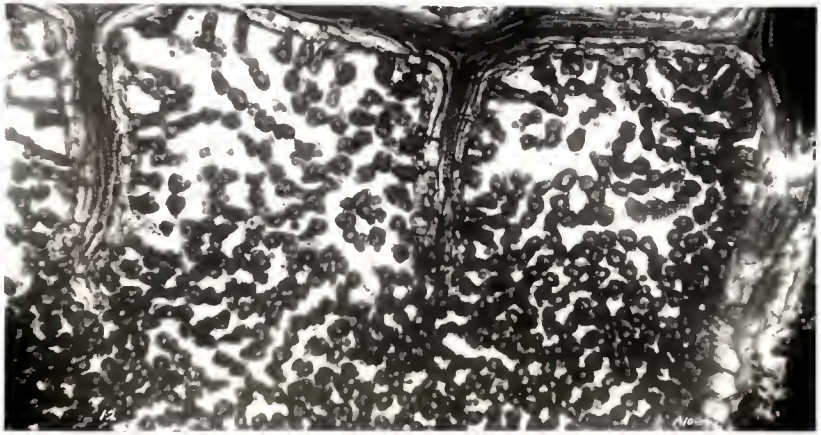

Fig. 30. Tangential section through the third layer of palisade, Wealthy sprayed greenhouse leaf (x315).

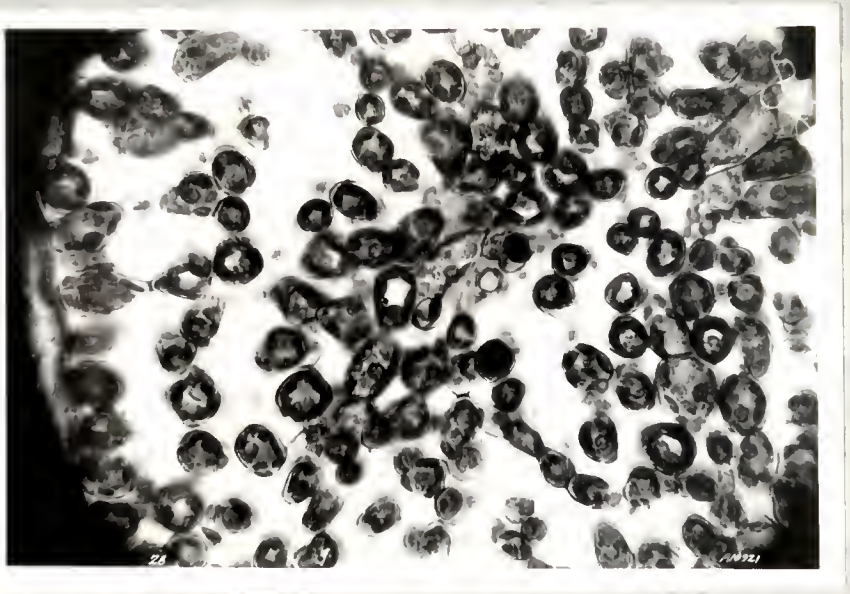

Fig. 31. Tangential section through the third layer of palisade, Wealthy unsprayed greenhouse leaf (x700).

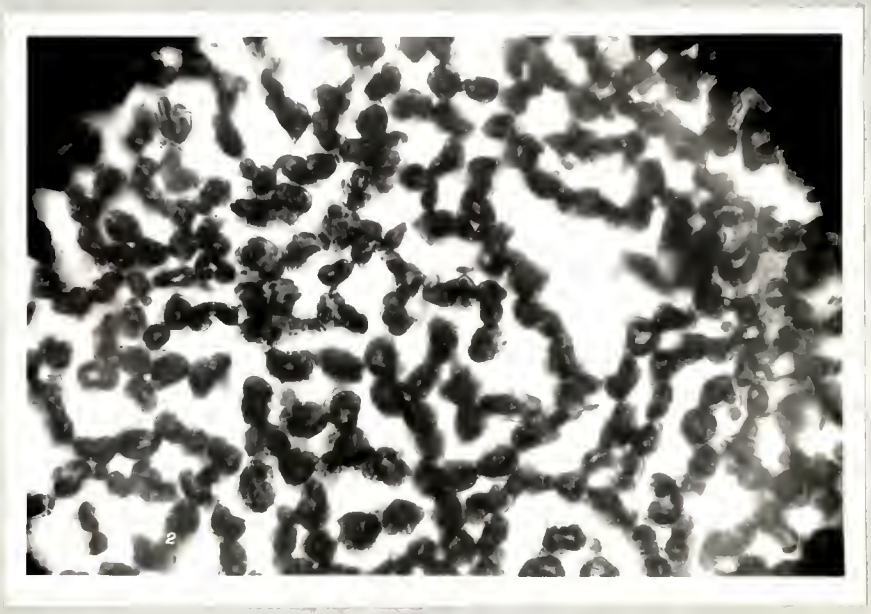

Fig. 32. Tangential section through the third layer of palisade, Wealthy sprayed greenhouse leaf (x700).

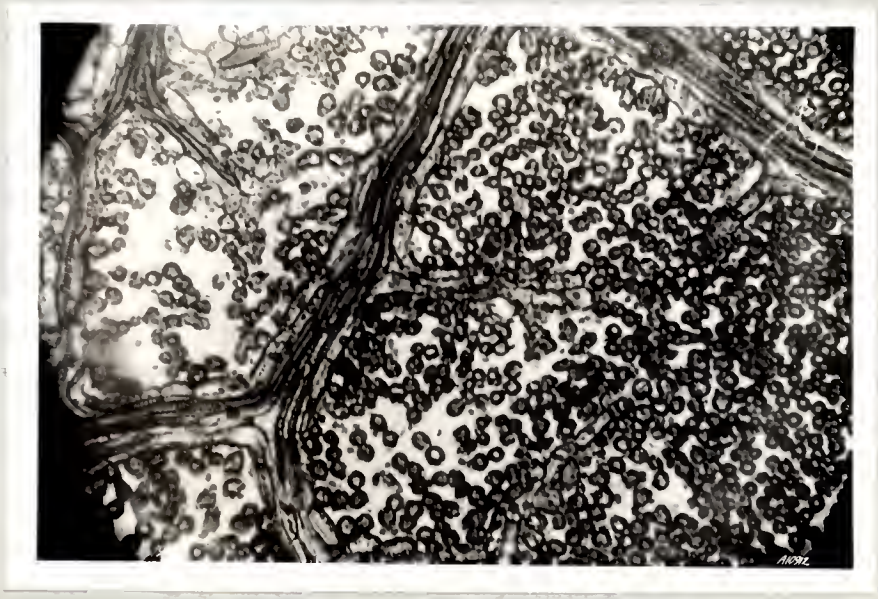

Fig. 33. Tangential section through the third layer of palisade, York unsprayed greenhouse leaf (x315).

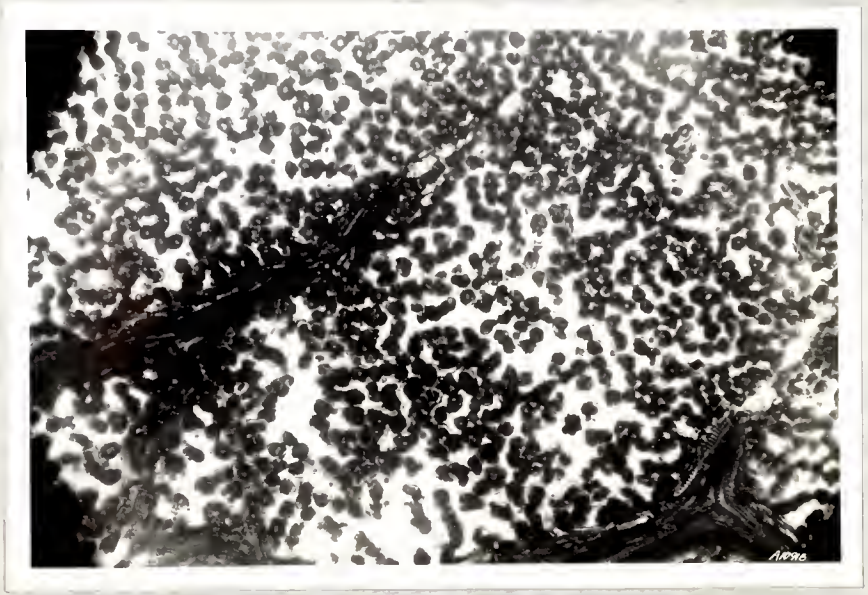

Fig. 34. Tangential section through the third layer of palisade, York sprayed greenhouse leaf (x315).

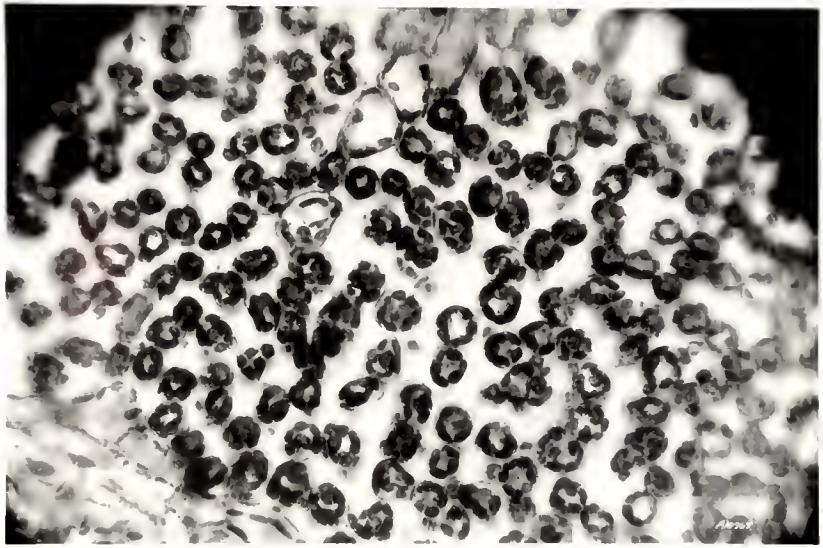

Fig. 35. Tangential section through the third layer of palisade, York unsprayed greenhouse leaf (x700).

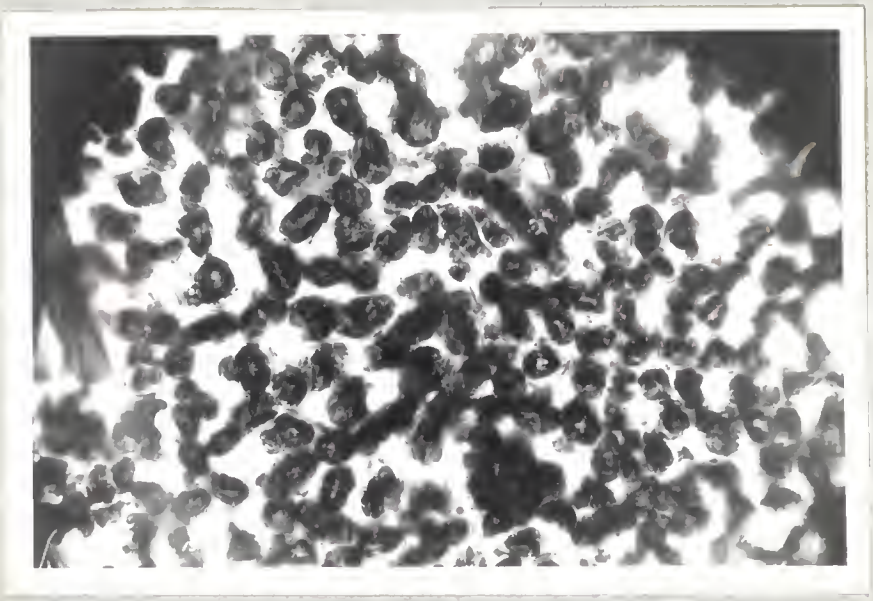

Fig. 36. Tangential section through the third layer of palisade, York sprayed greenhouse leaf (x700).

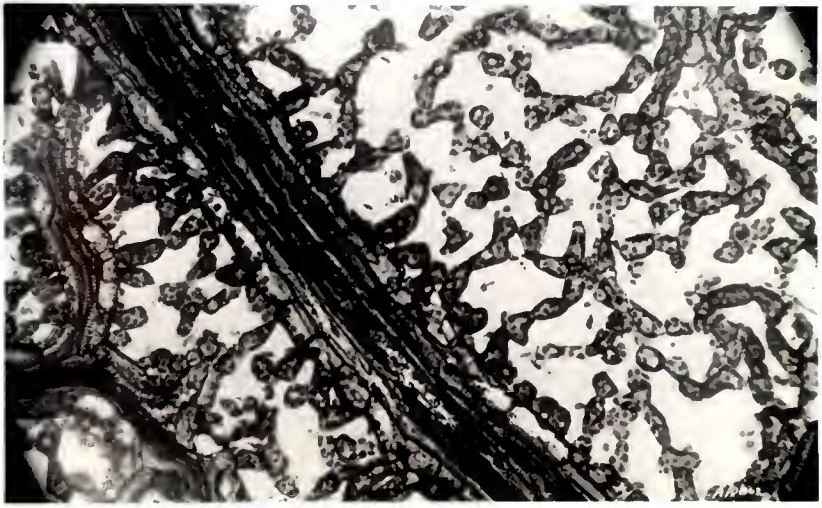

Fig. 37. Tangential section through spongy mesophyll of Wealthy unsprayed greenhouse leaf (x315).

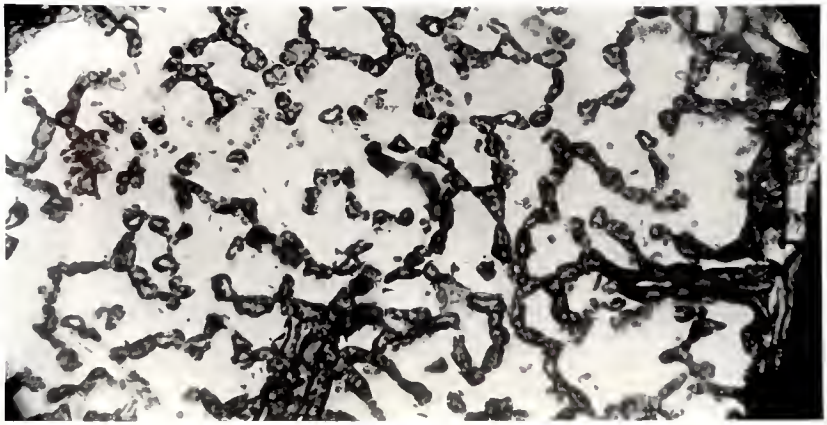

Fig. 38. Tangential section through spongy mesophyll of Wealthy sprayed greenhouse leaf (x315).

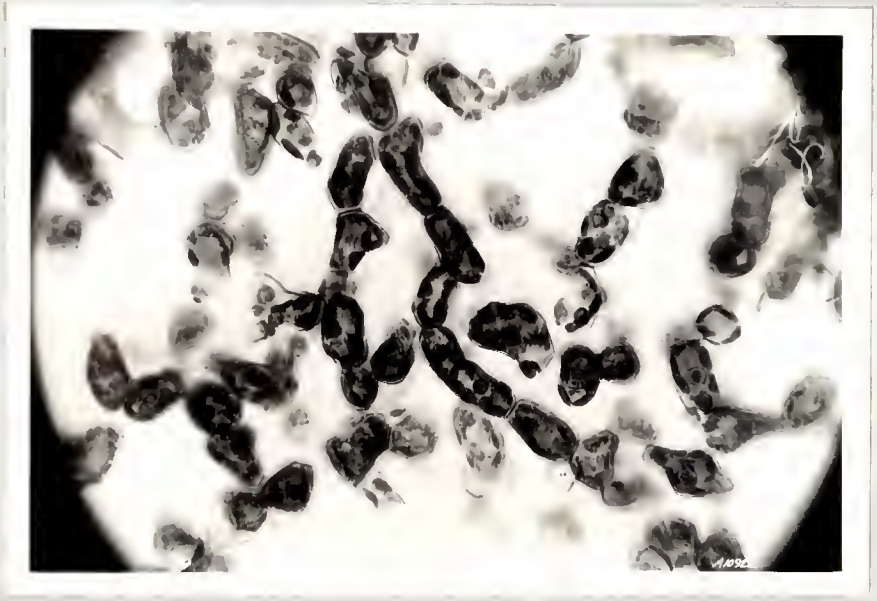

Fig. 39. Tangential section through spongy mesophyll of Wealthy unsprayed greenhouse leaf (x700).

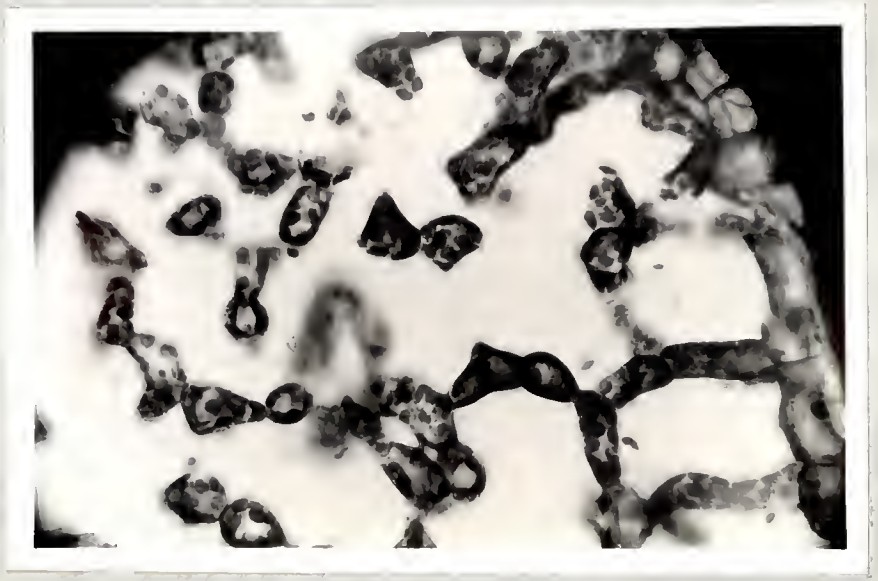

Fig. 40. Tangential section through spongy mesophyll of Wealthy sprayed greenhouse leaf (x700).

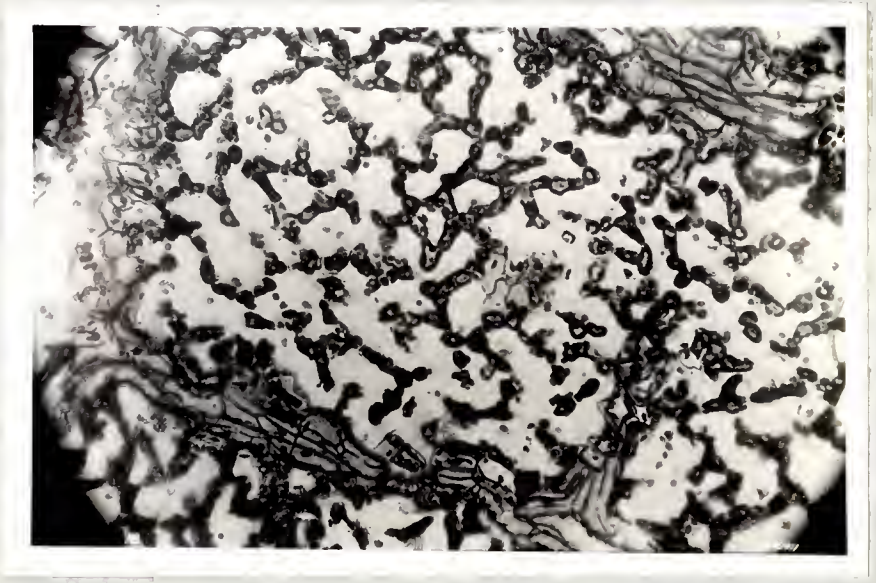

Fig. 41. Tangential section through spongy mesophyll of York unsprayed greenhouse leaf (x315).

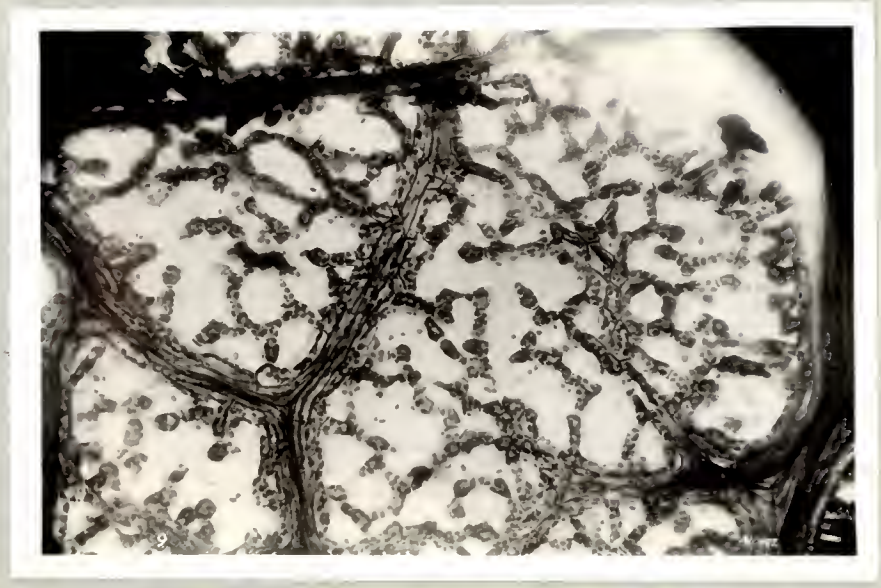

Fig. 42. Tangential section through spongy mesophyll of York sprayed greenhouse leaf (x315).

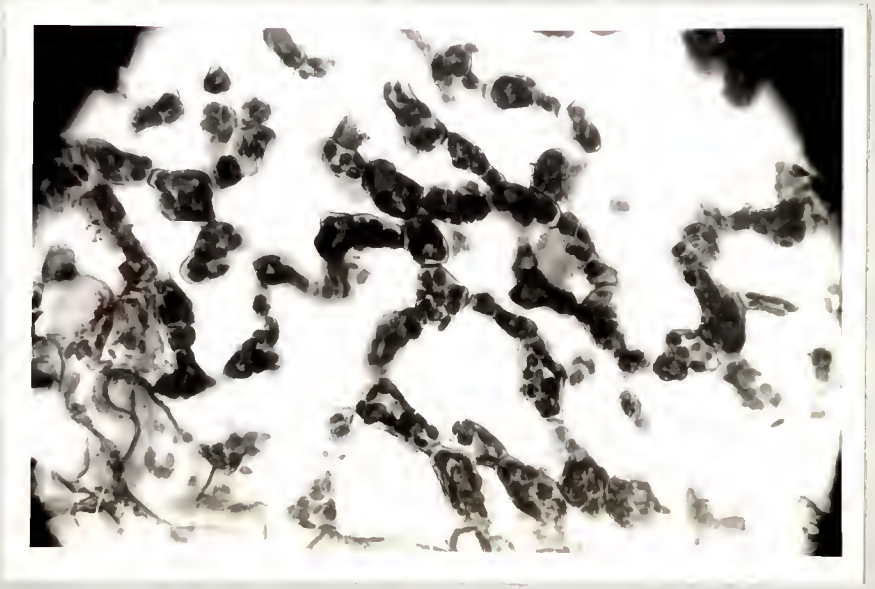

Fig. 43. Tangential section through spongy mesophyll of York unsprayed greenhouse leaf (x700).

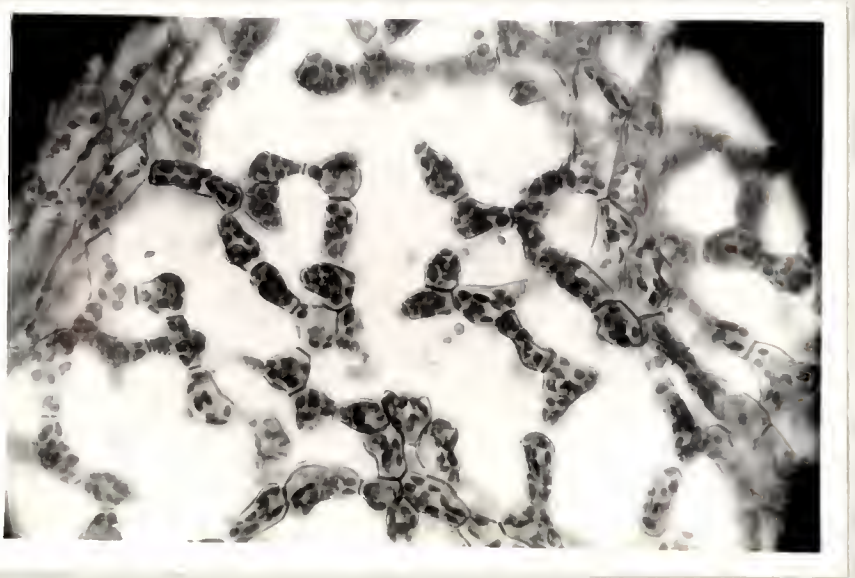

Fig. 44. Tangential section through spongy mesophyll of York sprayed greenhouse leaf (x700).

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